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LIFE CYCLE AND HOST-PARASITE RELATIONSHIPS OF *SCHISTOTAENIA TENUICIRRUS* (CESTODA: AMABILIIDAE)

by

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INTRODUCTION

The cestode, Schistotaenia tenuicirrus Chandler, 1948, is an intestinal parasite of the pied-billed grebe (Podilymbus podiceps L.) of Iowa, Minnesota, Michigan, Ohio, and Illinois. It has also been reported from the horned grebe, Colymbus auritus L., in the same areas.

Schistotaenia, together with the genera Amabilia Diamare, 1893, and Tatria Kowalewski, 1904, comprise the family Amabiliidae Ransom, 1909. No published accounts of life cycles of any members of Amabilia have appeared, and until the present study, no life cycles of members of Schistotaenia were known. Several accounts dealing with intermediate stages in the life cycles of members of Tatria, however, have appeared in helminthological literature.

Since pied-billed grebes of northwestern Iowa are known to be infected with Schistotaenia tenuicirrus, a study was undertaken in 1962-1965 to ascertain its life history and host-parasite relationships. Field collections and experimental studies were conducted at the Iowa Lakeside Laboratory in northwest Iowa. The discovery of the unusual intermediate stage of S. tenuicirrus, for which the term strobilocercoid is proposed, provides an additional example of the varied larvae known to occur among cyclophyllidean tapeworms.

HISTORICAL REVIEW

The genus Schistotaenia was created by Cohn (1900) to include small avian cyclophyllidean tapeworms presently assigned to the family Amabiliidae Ransom, 1909. Tapeworms of this genus are craspedote, possess conspicuous lateral outgrowths, and are parasites of grebes.

Because a thorough historical review and taxonomic revision of the family Amabiliidae was presented by Johri (1959), only a brief account is presented below.

Braun (1900) established the subfamily Amabiliinae of the family Taeniidae to receive the genus Amabilia Diamare, 1893. Later, Fuhrmann (1907) elevated the status of the subfamily Amabiliinae Braun, 1900, to that of a family, Amabilinidae, which was later correctly designated as Amabiliidae Ransom, 1909. Fuhrmann (1907) placed three genera in this family: Amabilia Diamare, 1893; Schistotaenia Cohn, 1900; and Tatria Kowalewski, 1904. The distinguishing characteristics of the family include the following: small cestodes with a strongly armed, tenacious rostellum; strobila with craspedote proglottids having conspicuous lateral appendages upon which the male genital pores open; genitalia single, alternating regularly or irregularly, or double; true vagina and vaginal pore absent and occasionally replaced in function by median accessory ducts opening to the exterior both dor-

sally and ventrally.

Johri (1959), noting the distinct differences between the characters of Amabilia and those of Schistotaenia and Tatria, proposed that the family Amabiliidae be divided into two subfamilies, Amabiliinae Braun, 1900, for the reception of the genus Amabilia Diamare, 1893, and Schistotiinae Johri, 1959, for the genera Schistotaenia Cohn, 1900, and Tatria Kowalewski, 1904. The subfamily Schistotiinae was changed to Schistotaeniinae by Owen (1960), so as to conform to the International Code of Zoological Nomenclature.

The unique features of the subfamily Amabiliinae enumerated by Johri (1959) include the following: double male genital organs and double male genital pores present in each segment; cirrus spiny; a single and median set of female genital organs; ovary and vitelline gland dendritic, the latter much larger than the ovary. Adult worms are found in the flamingo.

The distinguishing characters of the subfamily Schistotaeniinae include the following: single male genital organs and genital pores in each segment, alternating regularly or irregularly; cirrus spiny or non-spiny; female genital organs single in each segment and median in position; ovary bilobed, and larger than the vitelline gland; true vagina absent; seminal receptacles consisting of thin-walled sacs in the midline and communicating with one another in consecutive seg-

ments. Adult worms occur in the intestine of grebes.

The genus Schistotaenia Cohn, 1900, is distinguished from the closely related genus Tatria Kowalewski, 1904, by the presence, in the former, of numerous testes and of irregularly alternating male genital pores. Dorso-ventral canals replace the true vagina.

Six species are known for the genus Schistotaenia Cohn, 1900, namely: S. macrorhyncha (Rudolphi, 1810) Cohn, 1900; S. scolopendra (Diesing, 1856) Baer, 1940; S. macrocirrus Chandler, 1948; S. tenuicirrus Chandler, 1948; S. colymba Schell, 1955; and S. indica Johri, 1959. The significant characters for separating these six species are summarized in Table 1. Mr. John Gallimore, Department of Zoology, University of Alberta, Edmonton, Alberta, Canada, has informed me (personal communication) that he has found a new, as yet undescribed, species of Schistotaenia.

The present report involves the life cycle of one of these species, S. tenuicirrus, a common parasite of pied-billed grebes of northwest Iowa. The adult worm was named and described thoroughly by Chandler (1948). No life cycle studies on any members of the genus have appeared prior to an abstract by Boertje and Ulmer (1965).

Wardle and McLeod (1952) have suggested that the genera of the family Amabiliidae are indiscriminately and unsatisfactorily grouped according to aberrant features, and that

the discovery of new material or a closer re-examination of existing material may entirely change the present system of classification. Lopez-Neyra (1953) expressed the belief that the family Amabiliidae is an unnatural group based on teratological specimens. Matevosyan and Okorokov (1959), however, disagreed with this interpretation by Lopez-Neyra and considered Tatria a valid genus. The experimental studies on adults of S. tenuicirrus presented in this study, as well as the peculiar larval stage (strobilocercoid) involved in its life cycle, indicate that these cestodes, together with other amabiliids, probably constitute a unique group which should, for the present at least, be retained.

MATERIALS AND METHODS

Definitive Hosts

Most of the pied-billed grebes collected for this investigation were shot in ponds, sloughs, and lakes near the Iowa Lakeside Laboratory in northwest Iowa during the months of May through August of 1962-65. A 12-gauge automatic Remington shotgun was used for obtaining juvenile and adult birds. All grebes were examined for parasites within one hour of the time of death. Forty-three wild grebes were examined for the presence of helminths during this study.

For experimental purposes, eggs of pied-billed grebes were collected from nests in the spring of 1963 and 1964. From eggs incubated at approximately 38 degrees centigrade, four juveniles were hatched. A diet of boiled crayfish successfully kept two birds alive until after exposure to larval cestodes. Live crayfish were collected from adjacent rivers and ponds with seines, dip nets, and traps. By means of refrigeration, these crustaceans were preserved for several weeks.

During the spring of 1965, a wild juvenile grebe, estimated to be two or three days old, was caught by hand and was also used as an experimental host. The entire diet of this bird consisted of living crayfish. A wire cage (24" x 24" x 24") was designed for rearing the young grebes. A portion of

the floor of the cage was elevated 5" to the height of two plastic water basins (12" x 14" x 5" high), which were placed within the cage.

Laboratory-raised mallard ducks and wild grackles were also used for experimental exposures to strobilocercoids in studies concerning host specificity of Schistotaenia.

For collecting the internal parasites of grebes, the body cavity was opened ventrally, the digestive tract removed, and the viscera washed in 0.8% sodium chloride solution. The proventriculus and ventriculus were examined to determine the bird's food habits, as well as to check for the presence of larval parasites. An incision of the small intestine was made to obtain tapeworms for this study. To facilitate release of scoleces from the gut wall, sections of the host intestine with attached Schistotaenia were placed in Petri dishes with avian Ringer's solution and examined under the dissecting microscope. Tapeworms were relaxed in distilled water before fixation. Following fixation in AFA (alcohol, acetic acid, and formalin), the worms were stored in 70% ethyl alcohol. Mayer's paracarmine with a fast green counterstain was employed for staining all whole mounts, which were then cleared in xylene or methyl salicylate, and mounted in a synthetic resin (Permount). Tapeworms in situ were embedded in paraffin and cut at 10 to 12 microns on a rotary microtome. Stains utilized for these sections were

Mallory's triple or Harris's hematoxylin followed by an eosin counterstain.

Experiments involving the exposure of the eggs of Schistotaenia to various arthropods required the removal of gravid proglottids from adult worms. Some of these were fed immediately, others were kept from one to five days in distilled water and then fed to suspected intermediate hosts.

In other experiments, gravid proglottids were teased apart with dissecting needles to remove intra-uterine eggs. These were stored in filtered, boiled lake water or in distilled water, kept under refrigeration or at room temperature for periods of one to ten days, and the culture water changed daily. Eggs were then transferred to Petri dishes or Syracuse watch glasses for exposure to various invertebrates. An Eberbach shaking machine was employed for agitation of some eggs during incubation. A higher percentage of fully developed eggs was obtained with aeration and agitation at room temperature.

Identifications of birds were verified with the use of Birds of America, Pearson (1936), A Field Guide to the Birds, Peterson (1963), and the Handbook of North American Birds, Palmer (1962).

Intermediate Hosts

Many aquatic invertebrates near the Iowa Lakeside Laboratory were examined for larval stages of cestodes. Some of the arthropods collected were exposed to the eggs of Schistotaenia in an attempt to determine the intermediate host of this tapeworm. When the peculiar strobilocercoid larvae of Schistotaenia were found to occur in dragonfly naiads, many collections of these insects were made and the body cavities examined for larval stages with the aid of a dissecting microscope.

Naturally infected dragonfly naiads were collected from Jemmerson Slough, Marble Lake, Hale's Slough, and Prairie Lake in Dickinson County, Iowa. The most efficient collecting device for obtaining dragonfly naiads from vegetation was a long-handled bottom net with a rectangular bag 18" x 8" x 10" deep and a 6' handle of 1 1/4" diameter. Naiads were placed in separate containers in the laboratory, killed by removing the head, the hemocoel opened with dissecting needles, and the contents examined.

Dragonfly naiads for experimental studies were collected from the Big Kettle Hole located about six miles southwest of the Iowa Lakeside Laboratory. No grebes were observed on this pond, and no larval cestodes of Schistotaenia were ever recovered from 162 dragonfly naiads examined for naturally

occurring larval cestodes. Because of their cannibalism, these experimental hosts were isolated in finger bowls and small bottles. The amphipod, Hyallela azteca, collected from the Big Kettle Hole, was used as the primary food source.

Larval stages of Schistotaenia were fixed in AFA for whole mounts and in Bouin's for sections. Whole mounts were stained with Mayer's paracarmine and counterstained with fast green. Mayer's hemalum and Delafield's hematoxylin were used for staining sections. These were counterstained with eosin.

Crustaceans and insects were identified using keys by Needham and Westfall (1955), Pennak (1953), and Ward and Whipple (1959). Verification of the correct determination of the dragonfly naiad, Anax junius, was by Dr. Jean L. Laffoon, Department of Zoology and Entomology, Iowa State University.

Drawings were made with the use of a Leitz microprojector and a camera lucida.

SUMMARY OF LIFE CYCLE

The pied-billed grebe, Podilymbus podiceps, serves as the definitive host for Schistotaenia tenuicirrus in northwest Iowa. Natural infections in juvenile birds are considerably higher than in adult grebes. Sexually mature birds show a characteristic periodicity for the presence of adult worms; infected adult grebes are commonly found in May and June, but they lose their Schistotaenia infections during or soon after the nesting season.

The dragonfly naiad, Anax junius, serves as the intermediate host for the unusual strobilocercoid larva of S. tenuicirrus. Dragonfly infections are strictly seasonal, never occurring before July.

Embryonated eggs of S. tenuicirrus require an incubation period of five days before they are viable. Under experimental conditions, Anax junius naiads become infected with the larvae of S. tenuicirrus after exposure to embryonated eggs. The larvae occur free within the hemocoel 48 hours post-exposure. Rapid growth and development follows; in 21-day larvae, the scolex and strobila have very nearly completed development and strobilization occurs. The strobilocercoid larva is fully developed 28 days post-infection, and measures approximately 20 mm by 3 mm. Two distinct membranes surround the strobilocercoid, the inner one possessing

spirally arranged papillae, which constitutes one of the most distinctive features of the fully formed larva.

Experimental feedings of naturally infected dragonfly naiads containing strobilocercoids of S. tenuicirrus to laboratory-raised grebes conclusively establish the manner in which these birds acquire their infection. Gravid worms appear within 33 days post-exposure.

ADULT

General Statement

The pied-billed grebe, Podilymbus podiceps, serves as the principal definitive host for S. tenuicirrus, which has also been reported from the horned grebe (Colymbus auritus) and from the crow, Corvus brachyrhynchos, by Chandler (1948).

Previous investigations by Jones (1929), Baer (1940), and Chandler (1948) indicate that four distinct species of Schistotaenia parasitize the pied-billed grebe, namely, S. macrorhyncha, S. scolopendra, S. macrocirrus, and S. tenuicirrus.

Characters used in differentiating species of Schistotaenia include number, shape, and size of rostellar hooks; shape and size of scolex; diameter and form of the suckers; presence of spines on rostellum; shape and length of strobila; number of testes per segment; size of cirrus and cirrus pouch; regular or irregular alternation of male genital pores; size and form of seminal receptacles. Comparative measurements, principal hosts, and geographic distribution of the adult worms of the six known species of Schistotaenia are given in Table 1. Data for S. tenuicirrus are derived both from Chandler (1948) and the present study.

During the present investigation, 134 mature adults were collected and examined from naturally infected hosts. Some

Table 1. Comparison of characters of known species of *Schistotaenia*

	S. tenuicirrus	*S. macrorhyncha*	*S. scolopendra*
Strobila, length	20-96 mm	40.5 mm	7-11 mm
Scolex, length	0.6-1.8 mm		0.9 mm
Scolex, diameter	0.9-1.6 mm		0.325-0.44 mm
Sucker, diameter	0.24-0.3 mm 0.3-0.39 mm	0.16-0.21 mm	0.12-0.28 mm
Rostellar spines	present	present	present
Number of rostellar hooks	26	24-26	20
Hook, length	135-142 μ	145-162 μ	150-154 μ
Base of hook, length	115-125 μ	158 μ	124 μ
Alternation of male genital pores	irregular	irregular	irregular
Number of testes per segment	45-58	38-40	16-22
Cirrus, length	1.5 mm	0.94 mm	
Cirrus pouch, length	0.15-0.24 mm	0.14-0.16 mm	0.09-0.1 mm
Cirrus pouch, diameter	0.09-0.11 mm	0.1-0.12 mm	0.07-0.08 mm

Table 1. (continued)

	S. tenuicirrus	*S. macrorhyncha*	*S. scolopendra*
Seminal receptacle, diameter	0.12-0.15 mm		
Principal hosts	*Podilymbus podiceps* *Colymbus auritus*	*Podiceps nigricollis* *Podilymbus podiceps* *Colymbus auritus* *Colymbus dominicus*	*Podilymbus podiceps* *Podiceps dominicus*
Locality	Minn., Mich., Ohio, Ill., Iowa, Georgia	Europe, Texas	Antigua, South America
Reference	Chandler (1948); Boertje (present account)	Cohn (1900)	Baer (1940)

	S. macrocirrus	*S. colymba*	*S. indica*
Strobila, length	11-14 mm	42-46 mm	16-30 mm
Scolex, length	0.84-0.98 mm	1.04-1.06 mm	1.0-1.07 mm
Scolex, diameter	0.95-1.22 mm (width)	0.74-0.75 mm	1.13-1.46 mm (width)
Sucker, diameter	0.265-0.31 mm	0.234-0.249 mm	0.3-0.32 x 0.35 mm
Rostellar spines	absent	absent	absent

Table 1. (continued)

	S. macrocirrus	S. colymba	S. indica
Number of rostellar hooks	22	23	20
Total hook, length	125-130 μ	108-110 μ	130-170 μ
Base of hook, length	110-115 μ	84-87 μ	90-120 μ
Alternation of male genital pores	irregular	regular	irregular
Number of testes per segment	46-50	44-52	30-34
Cirrus, length	3.5 mm		0.4-0.5 mm
Cirrus pouch, length	0.34-0.38 mm	0.37-0.38 mm	0.37-0.4 mm
Cirrus pouch, diameter	0.19-0.21 mm	0.11-0.12 mm	0.095 mm
Seminal receptacle, diameter	0.7 mm		0.24 mm
Principal hosts	Podilymbus podiceps	Colymbus auritus	Podiceps ruficollis
Locality	Ohio	Idaho	India
Reference	Chandler (1948)	Schell (1955)	Johri (1959)

of the morphological differences of these specimens from Iowa, as contrasted with those described by Chandler (1948) from various Midwestern states, include length of strobila, size of scolex, size of rostellum, diameter of suckers, size of cirrus pouch, number of testes, and width of seminal receptacles. These are discussed below, and emendations relating to this species appear in the discussion section.

Strobila

The total length of the strobila of gravid S. tenuicirrus (Figures 1, 2) varies from 20 to 96 mm, with an average length of 35 mm. The maximum number of proglottids per worm is approximately 200. Chandler (1948) indicated his specimens to be from 20 to 30 mm long. It is rather surprising that he used body length as a key character, since in an earlier paper (1939), he showed that such factors as method of preparation, state of relaxation at time of death, age of worms, and number per host, had marked effects in determining the size of tapeworms. Of 212 adult S. tenuicirrus examined during this investigation, 91 were over 30 mm in length.

A distinct neck is present (Figure 6), and segmentation begins a short distance behind the scolex. All proglottids of the strobila are wider than they are long, and increase in length posteriorly. Proglottids are narrowest immediately behind the scolex, and their lateral margins are parallel for

most of the length of the worm. Each proglottid has a contractile, finger-like, lateral appendage on each side, measuring from 0.6 to 1.0 mm in length. Mature proglottids (exclusive of these contractile extensions) measure 0.35 mm long by 1.7 mm wide; gravid proglottids are 0.4 mm long by 1.85 mm wide.

The muscles (Figure 9) of the strobila are strongly developed. Under the cuticle and basement membrane lies a narrow region of subcuticular muscles separated by parenchyma from the underlying parenchymal (medullary) muscle fibers. The longitudinal muscles of the medullary region are not arranged in distinct layers, as described by Baer (1940) for S. scolopendra.

Circular or transverse parenchymal muscles, also well developed, form a single muscle layer. Dorso-ventral muscles are scattered through the medullary region between the reproductive organs.

The cortical parenchyma contains a large number of calcareous bodies, which also occur in the lateral appendages.

Scolex

The scolex (Figure 6) is large, 0.6 to 1.8 mm long, and posteriorly (at its widest portion) 0.9 to 1.6 mm in diameter. The narrowest region, immediately behind the rostellar hooks, has a diameter of 0.3 to 0.47 mm, depending on the state of

contraction. Great variations in the shape and size of the scolex (Figures 3-5) occur in accordance with the state of contraction of this region. The four spinose muscular suckers are spherical or oval and measure .24 to 0.3 mm by 0.3 to 0.39 mm in diameter. In young worms (Figure 30), suckers are much smaller.

The rostellum, large and heavily muscularized, resembles very closely that described by Cohn (1900) for S. macrorhyncha. Two muscular rostellar sacs (Figures 6, 7) are present, one inside the other. These unite anteriorly and are termed inner and outer rostellar sacs. The inner sac, about 0.4 to 0.46 mm long by 0.2 to 0.3 mm wide, consists of two muscle layers (Figure 7), a thick inner circular layer and an outer longitudinal layer. The outer muscular sac (about 0.8 mm long) consists of an inner circular and an outer longitudinal muscle layer. Some of these longitudinal muscles extend from the scolex into the strobila, are continuous with the longitudinal parenchymal muscles, and serve as retractor muscles. Additional extensions of the longitudinal body muscles (inner retractors, according to Cohn (1900)) penetrate the rostellar sac posterolaterally, extend anteriorly between the outer and inner rostellar sacs, and insert at the anterior rostellar region. They appear as four distinct muscle bundles within the outer rostellar sac, and between them lie glandular masses, the rostellar glands. These stain deeply with hema-

toxylin and hemalum, as previously reported by Cohn (1900) for S. macrorhyncha and by Baer (1940) for S. scolopendra. The entire surface of the rostellum is covered with small spines, and the apex bears a single crown of 26 very large hooks. Each hook (Figure 8) measures 135 to 145 microns in total length, from proximal end of base to tip of blade; from proximal end of base to tip of guard, 115 to 125 microns; from tip of blade to tip of guard, 52 to 59 microns; and from the anterior margin of hook to tip of guard, 82 to 86 microns.

The extremely well-developed musculature of the scolex enables the adult to attach tenaciously to its host, so that the rostellar region is often lost if worms are not carefully removed.

Male Reproductive System

S. tenuicirrus is protandric, testicular follicles (Figure 10) appearing very early in proglottids posterior to the scolex, prior to the formation of the ovary. In mature proglottids (Figure 11) they are located dorsally in the posterior half of each proglottid, extend across each segment exclusive of lateral appendages, and are separated by the seminal receptacle and yolk gland into two lateral areas, designated the poral and aporal sides. Testes number from 21 to 26 on the poral side and from 24 to 32 on the aporal side. This is the largest number of testes for any of the six

presently described species of Schistotaenia. The testes are spherical or oval and vary considerably in size, measuring 31.5 by 31.5 microns to 70 by 100 microns. They disappear abruptly in subsequent proglottids where seminal vesicles no longer contain sperm cells.

Genital pores are lateral and alternate irregularly. A muscular cirrus pouch (Figure 11) forms early, measuring 0.15 to 0.24 mm long by 0.09 to 0.11 mm in diameter when the cirrus is withdrawn. After evagination of the cirrus, the pouch contracts to a small pear-shaped body, measuring 0.05 to 0.12 mm long by 0.08 to 0.09 mm in diameter. The evaginated cirrus is long and slender, attaining a maximum length of 1.5 mm. It has a diameter of about 30 microns at its densely spinose base, diminishing to about 10 microns in diameter for the greater extent of the delicate flagellum. Under high power, the latter is seen to be covered with extremely delicate, short spines not discerned by Chandler (1948) in his original description of the species.

A small seminal vesicle (Figure 10) appears at the proximal end of the cirrus pouch in young proglottids. In mature proglottids, the seminal vesicles (Figure 11) reach a maximum size of 0.26 to 0.39 mm long by 0.2 to 0.28 mm in diameter. Between the seminal vesicle and the cirrus pouch, a smaller intermediate or prostatic vesicle (Figure 11) develops; because of compression between these larger structures, the

prostatic vesicle varies considerably in shape and size, measuring 0.06 to 0.1 mm long by 0.1 to 0.14 mm in transverse diameter. In several segments posterior to those in which the cirrus is evaginated, the cirrus pouch, seminal vesicle, and intermediate vesicle disappear. All the previously mentioned male genital organs are normally absent from segments containing a well-developed uterus, as in S. colymba and S. macrocirrus. Although Clerc (1907) for S. macrorhyncha and Chandler (1948) for S. macrocirrus suggested that the sudden disappearance of the copulatory apparatus resulted from extrusion or having been torn out by the roots, no evidence to support this view could be seen in S. tenuicirrus.

Female Reproductive System

The ovary (Figure 11) is densely lobed and located on the ventral side, stretching across the anterior width of the proglottid. Its greatest width is 1.4 mm. Medially, the vitelline gland lies dorsal and posterior to the ovary, and is 0.2 to 0.35 mm in transverse diameter.

The most unusual structure of the female genitalia is the seminal receptacle (Figures 10, 11). This structure appears first in very young proglottids as an ovoid sac located at the anterior border and in the mid-line. In mature segments the seminal receptacles are vase-shaped, their widest portion lying medially in the posterior portion

of the proglottid. A narrower portion projects anteriad and communicates with the receptacle of the preceding proglottid by a thin-walled duct. Sagittal sections show the seminal receptacles and longitudinal ducts in a linear series. Consequently, spermatozoa may migrate throughout the entire strobila, as they do in the closely related genus, Tatria. The maximum width of the seminal receptacle is 0.15 mm, its length corresponding to that of the proglottids (0.27 to 0.4 mm in proglottids possessing filled seminal vesicles). A true vagina is absent. In copulation, cirri are inserted directly into the seminal receptacles, by way of small dorsal and ventral canals extending from the body surfaces to the widest portion of the seminal receptacle. These canals, known as accessory dorso-ventral vaginae, have been described by Fuhrmann (1907) and Baer (1940). In whole mounts, these canals appear as narrow slit-like openings (Figure 11).

The uterus first appears before maturation of the testes as a median transverse tube. Eventually it appears somewhat lobulated and finally fills the entire medullary region of the gravid proglottid (Figure 12). The uterus ultimately becomes completely packed with embryonated eggs, described in another section of this paper. In terminal gravid segments, the lateral appendages begin to degenerate noticeably.

Related Species

According to Chandler (1948), S. tenuicirrus differs from S. macrocirrus in the shape and greater length of the body, larger rostellar hooks, smaller cirrus and cirrus pouch, and in shape and smaller size of the seminal receptacles. Results of the studies herein reported confirm Chandler's account.

From the description of S. scolopendra by Baer (1940), S. tenuicirrus may be distinguished by a larger and differently shaped scolex, greater number of hooks, greater length of the strobila, larger number of testes, and a larger cirrus pouch.

S. macrorhyncha Cohn (1900) is differentiated from S. tenuicirrus on the basis of hook size and shape, and on its geographic distribution. S. macrorhyncha has hooks with a relatively short blade, the hook length being 2 to 5 microns less than the base. It is considered an Old World species according to the key of Chandler (1948). However, Jones (1929) reported Podilymbus podiceps as a new host, and the state of Texas as a new locality for this species.

The two remaining species of Schistotaenia, which are not reported from the pied-billed grebe, are S. colymba and S. indica. Schell (1955) reported and described S. colymba from the horned grebe, Colymbus auritus, in Idaho. He indi-

cated major differences between this new species and S. tenuicirrus as the following: S. colymba has a scolex that is longer than it is wide, smaller hooks, smaller suckers, longer cirrus pouch, same number of testes in both halves of the proglottid, and genital pores alternating regularly instead of irregularly. In regard to the last characteristic noted, Schell's observations appear to be contrary to those of all other workers with reference to this important generic character of Schistotaenia.

Johri (1959) reported S. indica as a new species of Schistotaenia found in the little grebe, Podiceps ruficollis, in India. The major distinguishing features are size, shape, and fewer rostellar hooks, fewer testes, and longer cirrus pouch.

EGG AND ONCOSPHERE

Eggs of Schistotaenia tenuicirrus were obtained from the uteri of gravid worms within the small intestine of pied-billed grebes in two ways, by removing intra-uterine eggs from gravid proglottids or by allowing proglottids to disintegrate in distilled water. The first method proved to be the more satisfactory.

Eggs were incubated under various conditions. Cultures were maintained in filtered, boiled lake water or distilled water for periods from 1 to 10 days. Incubation of the eggs was at room temperature (25°-27° C.) under daylight conditions or in unlighted, constant-temperature conditions of 4°, 10°, 15°, 20° C., respectively. Water was changed daily and the eggs examined under a compound microscope to detect any movement or change in development. One of the most difficult problems in this study was to acquire viable eggs which would be infective in the proper intermediate host. When it was found that eggs recently removed from adult worms were not viable, a shaking machine was utilized. Cultures were agitated and aerated at room temperature as suggested for the propagation of Spirometra by Mueller (1959). At the end of three days, active oncospheres were noted. Such activated eggs were then placed under refrigeration for later use or were used immediately for exposing dragonfly naiads.

Fully developed eggs (Figure 13) are produced within the uteri of worms within four weeks of the time of infection of the avian host. These were obtained from an experimentally infected, laboratory-raised grebe in 1963. However, previous daily examination of the feces showed no eggs or proglottids. The thin-shelled non-operculate eggs average 87 (85-89) by 93 (90-96) microns. They are transparent, yolkless, and each contains a typical hexacanth embryo. Although a detailed description of the eggs of the genus Schistotaenia has not appeared previously in helminthological literature, the membranes associated with the eggs of S. tenuicirrus appear to be similar to those associated with the eggs of other cyclophyllidean cestodes. The terminology used by various authors to describe such membranes has not been uniform, but that proposed by Smyth (1963) is followed here. The outermost membrane or capsule of the egg of S. tenuicirrus is thin, and beneath it is a "gelatinous layer" or vitelline layer. Underlying this layer is the relatively thick, non-striated embryophore. Within the embryophore lies the oncosphere (Figure 14), surrounded by a very thin, delicate oncospheral membrane. The oncosphere is spherical and averages 50 (46-54) by 53 (50-56) microns. Previous descriptions of Schistotaenia have included no reference to embryonic hooks. Six slender oncospheral hooks are clearly seen in S. tenuicirrus eggs even while in utero. The lateral hooks average 12

microns in length, whereas those of the somewhat longer median pair average 15 microns. Additional details of the internal structure of the oncosphere were not studied.

Eggs of S. tenuicirrus are apparently much larger than those reported for other species of Schistotaenia. Chandler (1948) reported eggs and oncospheres of S. macrocirrus to be 50 to 55 microns and 22 to 24 microns in diameter, respectively. Schell (1955) indicated that the eggs of S. colymba measure 18 microns in diameter, and that none were embryonated when removed from gravid proglottids. According to Johri (1959), eggs and oncospheres of S. indica measured 52 to 56 microns and 34 to 36 microns in diameter, respectively, and embryonic hooks were not visible within eggs removed from gravid worms.

INTERMEDIATE HOST

General Statement

Prior to the present study, the intermediate hosts for species of the cyclophyllidean genus Schistotaenia were unknown. During this investigation, strobilocercoids of S. tenuicirrus were found to parasitize naiads of the dragonfly, Anax junius Drury, 1773 (Figures 15, 16).

This is one of our commonest and most widely distributed dragonflies, among the earliest to appear in the spring, and one of the last to disappear before winter. The adult Anax junius, known as the "big green darner", has a robust, olive-green body with distinct trimmings of blue and brown. The face is yellow and appendages brown. The female Anax possesses an ovipositor adapted for cutting holes in the stems of aquatic plants where the eggs are usually laid.

The yellowish eggs, approximately a millimeter in length, require about three weeks for development, and the recently emerged naiad is tiny, long-legged, and scarcely a tenth of an inch in length. The smooth, slender body is at first pale green with dark brown longitudinal streaks. The depth of coloring varies with environment and age. The legs are long and fitted with strong tarsal claws. The lower lip or labium, by means of which the naiad secures its food, is a fine grasping mechanism. It may be rapidly extended to a

length nearly a fourth of the entire body. At its tip, the labium bears two lobes armed with powerful hooks. When a victim is seized, the lobes close and the labium is withdrawn, thus bringing the prey into a position where it may be torn by the powerful mandibles.

An Anax naiad will apparently devour any living organism that it is capable of handling. Naiads are cannibalistic and are extremely clever hunters. They cling to the stems of aquatic plants, preferably hanging head downward, and conceal themselves as much as possible. They remain motionless until prey comes within reach, and with a swift stroke of the labium capture the victim. They climb rapidly, and swim well by means of ejections of water from the tracheal gill chamber.

The rates of growth and duration of the nymphal life of Anax have been determined by Calvert (1934). Identification and differentiation of the 13 instars of Anax junius in this study were made by comparisons with his observations and measurements.

Other aquatic invertebrates in northwest Iowa were examined for the presence of strobilocercoids of S. tenuicirrus. Many dragonfly naiads of the genus Libellula, damselfly naiads, water boatmen, backswimmers, water scuds, and crayfish were exposed to the embryonated eggs of S. tenuicirrus during the summers of 1962 and 1963. No infections with cestode larvae of this species were established, however. Two naturally

infected water boatmen, Sigara sp., harboring small cysticercoids of an undetermined type, were recovered from Jemmerson Slough on August 13, 1963.

The only other genus included in the subfamily Schistotaeniinae is Tatria Kowalewskii, 1904. Various species of damselfly and dragonfly naiads have been reported as the intermediate hosts for cestodes of this genus. Linstow (1892) found cysticercoids of T. acanthorhyncha Wedl, 1855, in naiads of the damselfly, Agrion puella. A related species, T. decacantha, was reported by Yamaguti (1940) in dragonfly naiads of Pseudothemis zonata, Crocothemis servilia, and Anax parthenope. Golikova (1960) found larvae of T. decacantha in damselfly naiads of the genus Agrion. Observations on the life cycle of T. acanthorhyncha were published by Mrazek (1927). All known life cycles of members of the genus Tatria, however, involve small cysticercoids with no indication of any larval stage characterized by a distinctly strobilate body.

Location of Parasite

The strobilocercoid larva of Schistotaenia tenuicirrus is invariably found within the posterior portion of the hemocoel of the dragonfly naiad, Anax junius. The diaphragm of the Anax larva is a strongly developed sheet of transverse muscle fibers closely surrounding the posterior end of the

mesenteron and the ventral tracheal trunk between the 4th and 5th abdominal segments, and just anterior to the Malpighian tubules. The diaphragm thus divides the abdominal cavity into an anterior compartment continuous with the thoracic and head cavities, and a posterior compartment containing the respiratory chamber of the intestine. The larval cestode always develops in this posterior compartment, lying lateral to the intestine and the rectal gills of the dragonfly naiad (Figure 28).

From one to three larvae of S. tenuicirrus were recovered from natural infections. The larva (Figure 16) is unusually large and in multiple infections completely fills the hemocoel of the dragonfly naiad. Even in single infections, the strobilocercoid, because of its length, cannot be extended, but is doubled upon itself, taking on a horse-shoe shaped appearance.

Usually the exoskeleton of an infected dragonfly naiad is sufficiently translucent to permit observation of the internal, distinctly white strobilocercoid without dissection of the host. Immediately following ecdysis of the dragonfly, the degree of visibility of the larval cestode is greatly increased. To the naked eye, infected dragonflies may sometimes be easily distinguished from non-infected naiads due to the presence of the large chalky-white strobilocercoids.

The location of the strobilocercoid of S. tenuicirrus

within the hemocoel of its host corresponds to many other cyclophyllidean cestodes having an arthropod intermediate host and a cysticercoid cestode larva. Cysticercoids of the closely related genus Fatria, as noted above, have also been reported from the body cavity of dragonfly and damselfly naiads.

Natural Infections

During 1963 through 1965, 2,056 Anax junius naiads collected from lakes and sloughs in northwest Iowa were examined for larval helminths. A summary of these insects collected and those found to be parasitized with strobilocercoids of S. tenuicirrus is presented in Table 2. A total of 30 (1.5%) infected naiads were collected from areas where infected grebes are known to occur. Multiple infections of strobilocercoids were present in several of the parasitized insects. Two harbored triple infections, one contained two strobilocercoids, and all other infected naiads harbored a single larval cestode.

The percentage of infection never exceeded 14%. The 30 infected naiads constituted 2.3% of the total number (1,281) of dragonflies taken from areas of known larval infections.

A striking characteristic of the larva of S. tenuicirrus is its marked seasonal distribution in dragonfly naiads. The percentage of infection of A. junius with strobilocercoids

Table 2. Infection of dragonfly naiads (*Anax junius*) with *S. tenuicirrus* during 1963 through 1965

	Collection area	Number of *Anax junius* examined	Number infected with *S. tenuicirrus*
1963			
June	Jemmerson Slough	49	0
July	Jemmerson Slough	30	0
August	Jemmerson Slough	28	4 (14%)
September	Jemmerson Slough	32	1 (3%)
1964			
June	Yaeger Slough	16	0
	Jemmerson Slough	28	0
July	Jemmerson Slough	158	0
	Marble Lake	32	0
August	Jemmerson Slough	132	5 (3.8%)
	Prairie Lake	42	0
September	Prairie Lake	16	2 (13%)
	Jemmerson Slough	64	7 (11%)
October	Jemmerson Slough	55	1 (2%)
1965			
May	Jemmerson Slough	5	0
June	Hale's Slough	249	0
	Jemmerson Slough	396	0
July	Jemmerson Slough	605	4 (0.7%)
August	Jemmerson Slough	119	6 (5%)
Totals		2,056	30

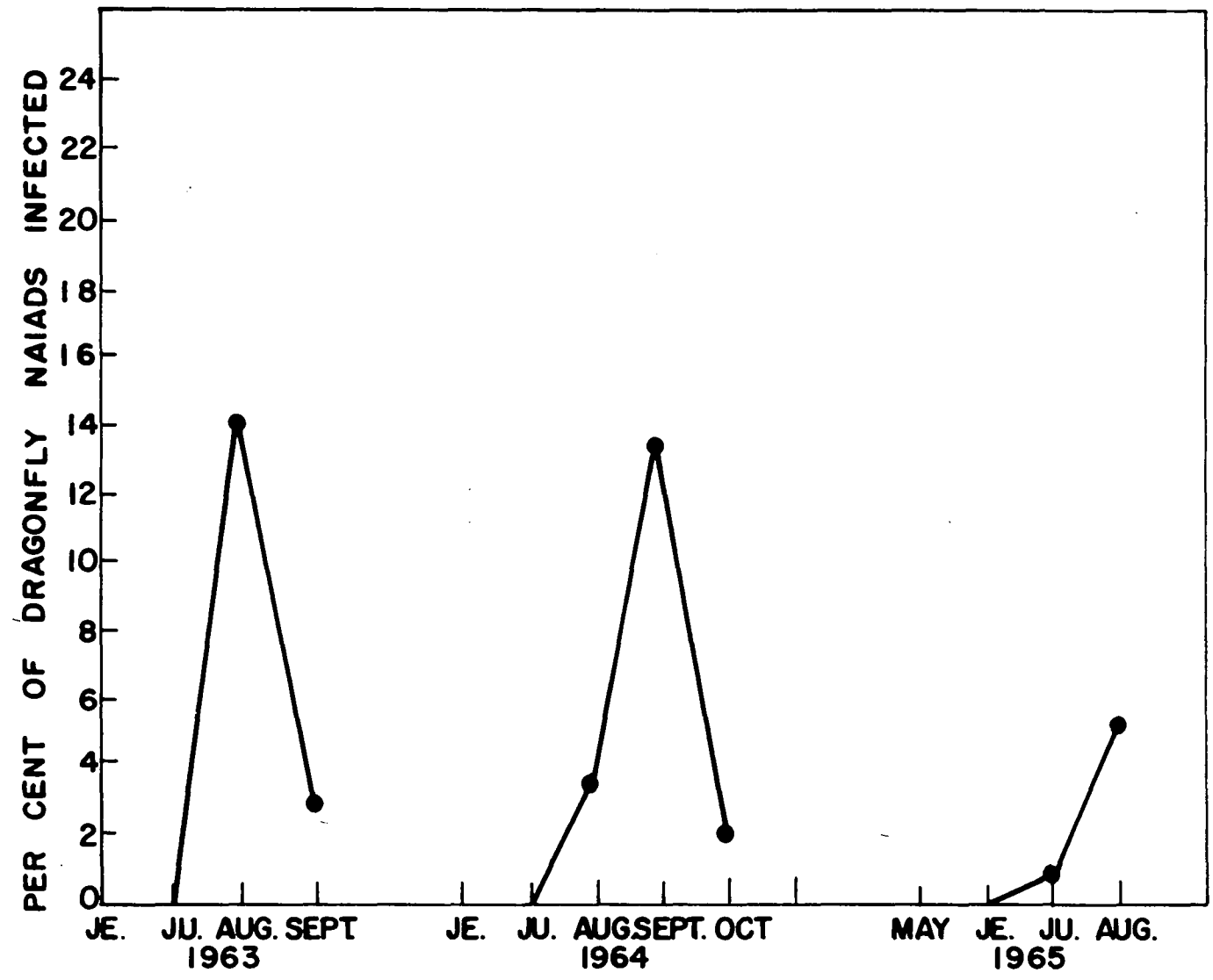
from areas known to harbor the infection during certain months of 1963 through 1965 is illustrated in Graph 1. No infected dragonflies were recovered during the months of May or June, indicating a distinct seasonal periodicity. During July of 1963 and 1964, of 220 dragonfly naiads examined, none were infected. However, during the month of July in 1965, 4 of 605 naiads harbored strobilocercoids. This was a very low percentage of infection: 0.7% compared to the higher average rate of infection of 5.8% for the months of August, September, and October 1963-1965.

These field studies show the complete absence of infected dragonfly naiads during the spring of each year. Insects apparently acquire their infections of larval S. tenuicirrus in early summer. Field collections indicate the appearance of strobilocercoids during the month of July, and their continued survival within the intermediate hosts until late October. Strobilocercoids do not appear to overwinter, as they were never found in early spring.

Pathology

Although fully developed strobilocercoids within the hemocoel result in death of the dragonfly naiad, early developmental stages of the parasite do not appear to interfere with normal growth and development of the host. From experimental studies it was observed that the parasite does not

Graph 1. Natural infections of *Anax junius* with strobilocercoids of *S. tenuicirrus*, 1963-1965


PER CENT OF DRAGONFLY NAIADS INFECTED
0
2
4
6
8
10
12
14
16
18
20
22
24
JE.
JU.
AUG.
SEPT
1963
JE.
JU.
AUG.
SEPT.
OCT
1964
MAY
JE.
JU.
AUG.
1965

interfere with the molting process during development from the 9th (the earliest natural infections encountered) to the 13th instars. Five naturally infected Anax junius naiads were continuously observed in the laboratory until death occurred during the 13th or final instar. Collection details and time of death are given in Table 3. Uninfected naiads of

Table 3. Summary of naturally infected A. junius naiads harboring strobilocercoids of S. tenuicirrus during 1965

	Observation number				
	1	2	3	4	5
Date of collection	Ju 13	Ju 20	Ju 22	Ju 22	Au 3
Length of naiad when collected	39 mm	36 mm	33 mm	39 mm	36 mm
Date of death of naiad	Ju 22	Au 26	Au 18	Sept 9	Sept 14
Length of naiad at time of death	41 mm	44 mm	44 mm	45 mm	43 mm

A. junius, according to Calvert (1934), normally metamorphose after having attained a length of 41.5 to 50 mm. As indicated in Table 3, infected naiads failed to metamorphose even after having attained this size. This is evidence that mortality of naiads due to the presence of strobilocercoids occurs only at the last instar. The parasitized naiads were

unable to undergo metamorphosis to the adult stage. Three of the five naiads expired after they had crawled from the water and after a split of the exuvia had occurred in this transition to the adult stage. The emerging adult was unable to free itself from the nymphal skin. The other two parasitized naiads died during the final instar before transformation to the adult form had begun. One of these two dragonfly hosts harbored a multiple infection of three strobilocercoids of S. tenuicirrus, and the other harbored a single parasite.

The very large size of the larval cestode suggests a need for considerable absorption of nutrients from the hemolymph of its host. However, this food is apparently adequately provided during the nymphal life since the naiad has a voracious appetite and is an extremely clever hunter. Nevertheless, the pathological effect on the final instar could very well be that of a nutritional deficiency. It is known from previous observations by Needham and Westfall (1955) that the naiad takes no food for several days before the time of transformation to the adult dragonfly. The nutritional requirements of the parasite at this critical period in the metamorphosis of the dragonfly could sufficiently weaken the host so as to produce a lethal result.

The great size of the fully-formed strobilocercoid may also produce seriously detrimental effects on the respiratory system of the dragonfly naiad, in that the larva may inter-

fere with the normal functioning of the branchial basket of its host. The rectal region acts as a diffusion membrane, allowing the passage of oxygen inward from the water to the tracheal capillaries. Interference with normal rectal respiration of the dragonfly at the critical period of metamorphosis from naiad to the adult may have serious consequences. It is doubtful that the presence of the strobilocercoid within the anisopteran naiad has a harmful effect on the functioning of the rectum in the excretion of the fecal pellets or in propulsion.

The death of the aquatic naiad, before it leaves the water for an aerial existence as an adult, is unquestionably advantageous to the survival of the parasite; it provides an effective method for the intermediate host to be ingested by the definitive host. The rapid flight of the adult dragonfly probably prevents it from serving as a common prey of the juvenile or adult grebe.

DEVELOPMENT AND MORPHOLOGY OF STROBILOCERCOID

Experimental infections of Anax junius naiads with the eggs of S. tenuicirrus were conducted with eggs obtained from worms occurring in naturally infected pied-billed grebes. Experimental exposures of cestode eggs to dragonfly naiads during the summer of 1964 produced negative results. However, during the summer of 1965, developmental stages of the strobilocercoid of S. tenuicirrus were recovered from experimentally infected A. junius naiads.

Gravid proglottids of S. tenuicirrus were fed directly to A. junius naiads on six occasions during June, July, and August of 1965. Gravid segments were fed immediately upon removal from the definitive host or were kept in distilled water from one to five days before feeding. Exposure was accomplished by moving a gravid proglottid (held by a forceps) in front of the dragonfly naiad previously starved for 3 to 4 days. The labium of the naiad was quickly extended to capture the proglottid, and ingestion immediately followed. However, none of these feeding experiments, summarized in Table 4, were successful in producing infections.

The use of embryonated eggs of S. tenuicirrus in producing infections proved more satisfactory. Eggs were obtained by teasing apart gravid proglottids in distilled water. Intra-uterine eggs, released in this way, were kept

Table 4. Summary of six feeding experiments conducted with *Anax junius* naiads and gravid proglottids of *S. tenuicirrus* in 1965

	Experiment number					
	1	2	3	4	5	6
Number of dragonfly naiads exposed	10	16	12	23	18	14
Proglottid incubation time, in days prior to feeding	0	1	5	2	3	4
Number of dragonfly naiads infected 2-3 weeks post-feeding.	0	0	0	0	0	0

under refrigeration or at room temperature for 1 to 10 days prior to use in feeding experiments. As indicated in a previous section dealing with the egg and oncosphere, agitation and aeration of some eggs at room temperature increased the percentage of fully developed eggs.

Feeding experiments were conducted by pipetting embryonated eggs into a Petri dish containing filtered, boiled lake water. Plastic dividers were used to provide eight individual compartments in each container. These barriers prevented cannibalism among the dragonfly naiads, but permitted circulation of the cestode eggs throughout the medium.

Prior to the exposure of dragonfly naiads to such embryonated eggs, naiads were starved for several days.

Immediately after exposure, amphipods (Hyalella azteca) were placed in the Petri dish so that naiads feeding on these aquatic organisms might accidentally ingest tapeworm eggs. Dragonfly naiads were exposed to the embryonated eggs for 24 hours, and were then removed from the Petri dish and placed in separate finger bowls.

During 1965, dragonfly naiads of the genera Libellula and Anax were exposed to embryonated eggs of S. tenuicirrus. Experimental feedings were attempted with 27 Libellula and 93 Anax naiads. Infections were established, however, only in A. junius.

In nature, 629 dragonfly naiads younger than the 9th instar were examined; none were found to harbor larvae of S. tenuicirrus. During experimental studies, infections of these earlier instars were also unsuccessful.

Results of five feeding experiments of embryonated eggs of S. tenuicirrus conducted with Anax junius (7th to 12th instar) in 1965 are summarized in Table 5. Although the number of infected insects is very low, it must be remembered that ingestion of eggs by naiads is apparently accidental and that some of the eggs may not have been viable. Results indicate that an incubation period of five days is necessary before eggs become infective.

The rectal tracheal gills of dragonfly naiads may very well have been involved in the intake of viable eggs. Onco-

Table 5. Summary of five feeding experiments conducted with embryonated eggs of S. tenuicirrus and the intermediate host, Anax junius, during 1965

	Experiment number				
	1	2	3	4	5
Number of dragonfly naiads exposed	24	18	15	12	24
Egg incubation time, in days, prior to exposure	6	2	4	10	8
Number of dragonflies infected	4	0	0	2	5
Number of days post-feeding	14-28	10-14	7-21	7-28	1-21
Number of multiple infections	0	0	0	1	2

spheres could then penetrate the gut and come to lie in the posterior portion of the hemocoel. If this mode of infection does occur, it would explain why no infections were successful by feeding proglottids directly.

Eggs of S. tenuicirrus were found in the intestine of exposed dragonfly naiads after two hours post-exposure. Young larvae, lying free within the hemocoel of the intermediate host, were first found after 48 hours post-exposure. Penetration of the intestinal wall by the oncosphere was not observed. The shape of the 48-hour larva (Figure 17) recovered from the hemocoel was spherical, approximately 60

microns in diameter; the three pairs of slender oncospheral hooks, located peripherally at one pole of the sphere, were still present. No cavity or structural differentiation of the internal cells was observed. This first stage corresponds in general to the level of development of cysticercoid formation established in earlier studies by Venard (1938) on development of the double-pored dog tapeworm, Dipylidium caninum, in studies of Wisseman (1945) on the fowl cestode, Raillietina cesticillus, and those of Voge and Heyneman (1957) on two species of hymenolepids, Hymenolepis nana and Hymenolepis diminuta in the intermediate host, Tribolium confusum.

A single 72-hour larva was recovered from the hemocoel of a dragonfly naiad on August 7, 1965. No discernible differentiation from the 48-hour stage was observed. The delicate larva was accidentally broken before measurements could be made. Unfortunately, intermediate stages of development between 72 hours and 2 weeks old were not available from experimentally infected naiads. However, subsequent development of the strobilocercoid apparently occurs very rapidly.

A single naturally infected dragonfly naiad captured on August 2, 1964, contained a strobilocercoid of unknown age, but its degree of development was intermediate to cestode larvae (72-hour and 14-day post-exposure) recovered from experimental hosts. This developing strobilocercoid measured

4 mm long by 0.8 mm in width. A longitudinal section (Figure 18) showed the presence of an outer sac, which at the posterior region, is continuous with the embryo proper. In later stages, however, this membrane is clearly separated from the embryo by a fluid-filled cavity.

Two 14-day larvae were recovered from two experimental hosts during 1965: one on June 25 and the other on July 26. These elongate larvae measured approximately 8 mm by 1.5 mm when recovered from their host. Fixed specimens (Figure 19) were somewhat smaller due to contraction at the time of fixation. An outer transparent covering or sac (Figure 21) encloses an opaque, greatly elongated whitish body, one end of which has partially differentiated into the definitive scolex. No evidence of strobilization appears in the 14-day larvae. The outer sac or "parenchymatösen Hülle" of Linstow (1892) is very elastic, almost constantly changing its appearance and shape through its contraction and elongation. It appears to be very similar to the outer covering of the larva of Tatria as described by Linstow (1892) and Mrazek (1927). An extensive fluid-filled cavity has developed between the outer membrane and the embryo proper. No evidence of oncospheral hooks could be discerned in this or in subsequent stages. Mrazek (1927) reported the larva of Tatria acanthorhyncha to be attached to the intestine of its host by a stalk, but no such attachment appears to be present in

Schistotaenia tenuicirrus. The origin of the outer covering was not determined in this study but warrants closer examination.

Anteriorly, the developing embryo is one of great cellular density. Development of the scolex and rostellum has occurred, these areas being clearly visible (Figure 19). Rostellar hook formation has already begun, and hooks appear to be soft, movable, and fingerlike. First indications of sucker development are shown by relatively dense lateral areas of the scolex primordium. Posterior to the scolex, the body appears to be filled with dense granular material. No segmentation was noted at 14 days. At the posterior end of the body is a minute invagination leading to a small cavity, similar to one described by Hendtorff (1948) in the developing larva of Oochoristica symmetrica, by Milleman (1955) in O. deserti, and by Hickman (1963) in O. vacuolata. Voge (1960), however, with reference to an apparently similar structure in the fully developed cysticercoid of Raillietina cesticillus, considers it merely as a "posterior fold" since its relationship to an excretory function has not been established. But longitudinal sections of the strobilocercoid of S. tenuicirrus show many excretory ducts in this posterior region and provide additional evidence of the existence of an excretory bladder.

On July 2, 1965, and August 25, 1965, two 21-day larvae

were recovered from two experimentally infected dragonfly naiads. Strobilization had occurred, and each larva consisted of approximately 8 segments. The scolex and strobila now constituted the embryo proper. These larvae had increased in size by approximately one-third and measured up to 12 mm in length. No additional structural differentiation of the outer sac could be observed. However, numerous granules, not observed in preceding stages, but present in all succeeding ones, were distributed throughout the fluid-filled cavity.

As a result of incipient withdrawal of the strobila and scolex (Figure 20), the embryo proper begins to become enveloped by a second (inner) membrane of the strobilocercoid. This membrane, continuous with the base of the strobila, exhibits on its outer surface a long spiral line extending throughout the length of the embryo. In fully formed strobilocercoids this spirally arranged configuration is highly modified by numerous papillae-like evaginations (Figure 24). In 3-week strobilocercoids, however, this spiral line shows only slight evidence of such evaginations.

The scolex and strobila have very nearly completed development in the 21-day larvae. The scolex shows increased motility, and also, contractions and expansions of the body are frequent. The rostellum and rostellar hooks are well differentiated and fully formed. Sucker musculature, espe-

cially the radial muscles, is distinct. The scolex and strobila evidently become well defined before withdrawal. This pattern of growth and differentiation of the scolex before invagination corresponds to that described for cysticercoids of Hymenolepis (Alicata and Chang (1939), Voge and Heyneman (1957)), Raillietina (Wisseman (1945)), and Oochoristica (Milleman (1955), Hickman (1963)).

Fully developed 28-day strobilocercoids were recovered from three experimentally infected hosts. Two infected naiads were examined on July 9, 1965, and two strobilocercoids were recovered from a single host on August 9, 1965. These, in all respects, were similar to fully formed strobilocercoids from naturally infected naiads (Figure 23). The mature larva is very large, long and slender. It is approximately 20 mm long and 3 mm broad in the central region and tapers to a point at each end. Its transparent, muscular, external sac, highly variable in shape due to muscular movements, surrounds a fluid-filled cavity containing a number of calcareous granules. The spirally arranged papillae (Figure 24) associated with the greatly twisted internal membrane (Figure 22) constitutes one of the most distinctive features of the fully formed larva. The withdrawal of the scolex and strobila leads to the formation of a long narrow tube (invagination canal), which connects the embryo proper and the anterior end of the inner membrane (Figures 24, 27). The combined

length of the scolex and strobila is now 2 to 4 mm and varies in width from 0.6 to 0.8 mm (Figure 25). The scolex is from 0.6 to 0.87 mm long and 0.75 to 0.9 mm broad across the posterior region. The rostellum may be retracted or evaginated from the rostellar sac. The muscular rostellum, armed with a single row of 26 hooks, is 0.35 to 0.42 mm long; the length of the rostellar sac is 0.52 to 0.63 mm. The suckers (Figure 26) are about 0.28 to 0.3 mm in diameter. Measurements of the rostellar hooks of the mature larvae are similar to those of adult worms. Distinct strobilization has increased with usually 6 to 20 segments present, depending on the degree of maturity. Proglottids just behind the scolex are narrow and the development of the lateral appendages is distinctly visible on the posterior segments. This strobilization of the larval tapeworm while still encysted in the intermediate host is similar to that of Hydatigera taeniaeformis strobilocerci in rats and mice.

Hutchinson (1958) studied the growth of the larval stage of Hydatigera taeniaeformis in the liver of mice. His studies showed that, on the 30th day after infection, an invaginated cysticercus developed. This larva evaginated on the 42nd day, and by the 48th day a strobilocercus had formed.

The activity of the strobilocercoid when removed from surrounding membranes is very unusual. The rostellum and

suckers show a high degree of muscular contraction and relaxation. The strobilate portion also undergoes constant contraction and elongation, with the lateral appendages assisting in locomotion. Fully developed strobilocercoid bodies are similar in all aspects to the youngest Schistotaenia tenuicirrus adults from grebes (Figures 29, 30).

DEFINITIVE HOSTS

General Statement

The pied-billed grebe, Podilymbus podiceps L. (Figure 37), is the only grebe which is a summer resident of the Okoboji region of northwestern Iowa. During the breeding season, this species is found mainly in lakes, marshes, and sloughs having emergent vegetation and areas of open water.

Although members of the cestode genus Schistotaenia have been reported from grebes since 1900, only four of the presently known species have been associated with the pied-billed grebe. Jones (1929) found S. macrorhyncha in this avian host in Texas, although it had been previously reported from grebes in Europe. According to Baer (1940), S. scolopendra also parasitizes the pied-billed grebe in Antigua, one of the Leeward Islands of the British West Indies. Two additional species of Schistotaenia were described by Chandler (1948), who reported S. macrocirrus from Podilymbus podiceps in Ohio and S. tenuicirrus from pied-billed grebes of Minnesota, Michigan, Ohio, and Illinois. Neylans later (1952) described S. tenuicirrus from P. podiceps in Georgia.

During 1962 through 1965, a total of 43 pied-billed grebes in northwest Iowa were collected and examined for the presence of helminths. Two species of trematodes, Petasiger nitidus Linton, 1928, and Echinostoma sp., and five species

of cestodes, Ligula intestinalis Goeze, 1782, Hymenolepis lobulata Mayhew, 1925, Haploparaxis sp., Tatria duodecacantha Olsen, 1939, and Schistotaenia tenuicirrus Chandler, 1948, were recovered.

Avian hosts examined and the incidence of S. tenuicirrus infections are listed in Table 6.

Location of Parasite

Adult S. tenuicirrus are found only in the small intestine of adult and juvenile pied-billed grebes. According to other workers, all species of Schistotaenia occupy this region of the digestive tract of their avian hosts. Scoleces of all specimens are deeply embedded in the submucosa of the duodenum or the proximal end of the jejunum. The minimum and maximum distances of the cestodes from the ventriculus of the grebe were 4 and 12 inches, respectively.

Sexually mature adult grebes appear to lose these cestodes at the time of the nesting season or soon thereafter. Some of the birds examined at this time showed seriously damaged areas of the intestine but no Schistotaenia were present. Although other adult grebes harbored these cestodes, the worms were old, appeared very sluggish, and contained no eggs. In infected juvenile birds, however, worms were vigorous, very active, and possessed gravid proglottids. When cestodes from juvenile birds were placed in avian Ringer's

Table 6. Summary of pied-billed grebes examined, 1962-65

	Number examined		Number infected with S. tenuicirrus		No. worms per bird
	Juveniles	Adults	Juveniles	Adults	
1962					
July	3	1	3	0	3,5,9
August	3	3	3	1	1,3,7,10
1963					
June	1	2	1	2	1,1,9
July	7	2	7	1	1,2,2,4 5,6,6,7
August	5	0	5	0	1,1,5,12,27
1964					
August	2	0	2	0	2,36
1965					
May	0	1	0	1	18
June	2	6	0	2	2,5
July	3	2	3	2	1,2,2,5,11
Totals	26	17	24 (92%)	9 (52.9%)	

solution, scoleces and strobila expanded and contracted continually. The suckers and rostellum were particularly active.

Natural Infections

During examination of 43 Podilymbus podiceps collected from sloughs and lakes in northwest Iowa during the summers of 1962 through 1965, 33 (77%) were parasitized with S. tenuicirrus. Of 26 juvenile birds, 24 (92%) were infected, compared to only nine (52.9%) infected adult grebes out of 17 examined. In 1962, of 10 pied-billed grebes collected during the months of July and August, seven were infected with S. tenuicirrus and three were negative. Of the seven infected hosts, six were juveniles and only one was sexually mature. The average number of Schistotaenia per infected bird was five (1-10). The only infected sexually mature bird was taken on August 7, 1962, and is believed to have been reinfected with a single cestode after losing its former worms. The basis for this assumption was the presence of characteristic intestinal scars presumably caused by previous S. tenuicirrus, and the tapeworm present was gravid and very active, which is not characteristic of old worms. The three negative grebes were all sexually mature and the absence of Schistotaenia suggests a characteristic periodicity of adult worms during and after the nesting season. Possibly a change in feeding habits by the adults, such as preying on larger organisms, like crayfish, fish, and amphibians, would account for a low percentage of reinfection.

In June, July, and August 1963, of 17 pied-billed grebes examined, all were infected with S. tenuicirrus except one sexually mature adult collected on July 30. The small intestine of this negative bird displayed necrosis and several distinct scars indicating previous attachment of former worms. After the month of June, all infected grebes examined were juveniles except one sexually mature adult collected on July 26. This adult bird contained six S. tenuicirrus which ranged in length from 2 to 14 mm. The small size of these worms indicated that the infective larvae were ingested by the definitive host earlier in the same month. The number of worms present indicated that this bird's diet contained many dragonfly naiads. During 1963, the average number of Schistotaenia per infected grebe was six (1-27).

Only two juvenile grebes were examined in 1964 during the month of August. One bird was parasitized with two S. tenuicirrus; the other, with 36. This latter bird had the highest number of Schistotaenia recovered from a single host. This grebe was sexually immature, indicating that all parasites were acquired during the summer months, and that the larval parasite was indigenous in large numbers to this particular locality. Of the 36 worms, four were less than 10 mm in length, nine were from 11 to 20 mm, nine were from 21 to 29 mm, seven were from 30 to 40 mm, and seven were from 41 to 51 mm.

During the 1965 collections of 14 pied-billed grebes examined, three juveniles and five adults were infected with S. tenuicirrus. The average number of these cestodes per infected bird was six (1-18). The three infected adult grebes taken during May and June harbored gravid worms; however, during July, only immature Schistotaenia were recovered from two sexually mature birds. Of the six negative Podilymbus taken in the month of June, four were sexually mature and two were very young, estimated to be approximately 3 to 5 days old.

No relationship appears to exist between the sex of the grebe and the rate of infections of S. tenuicirrus. During this investigation, 14 (70%) of 20 females and 19 (83%) of 23 male pied-billed grebes were parasitized with this cestode.

From these collections, a significant characteristic concerning the age of the worms was observed. In all six of the infected grebes taken during the months of May and June, all 36 of the S. tenuicirrus were gravid. The average length of the 36 worms was 37 mm (26-96 mm). This was not observed during the other summer months of the year when immature, mature, and gravid worms were present. These data, together with evidence obtained from feeding experiments, suggest that the larval stage of S. tenuicirrus is not developed sufficiently during May and June to be infective in northwest Iowa. This conclusion is further supported by the absence of

natural infections of Anax junius with the strobilocercoid of S. tenuicirrus during these months.

Experimental Infections

Following the discovery of the peculiar strobilocercoid larva within the hemocoel of dragonfly naiads, three feeding experiments were designed to establish it as the larval stage of S. tenuicirrus. Seven eggs of Podilymbus podiceps were collected from Hale's Slough and were incubated on June 19, 1963. Of these seven eggs, only two hatched on June 23. Approximately a month later on July 20, one juvenile bird suddenly died. On August 26, the remaining laboratory-raised grebe was fed a single naturally infected dragonfly naiad harboring a strobilocercoid larva. Four weeks later, a single gravid S. tenuicirrus was recovered from the small intestine. This experimentally reared worm was 33 mm in length, and the strobila consisted of 162 proglottids. Previous examination of the feces of the definitive host for eggs and proglottids did not indicate the presence of this adult tapeworm.

After this initial, successful feeding experiment, another collection of grebe eggs was made on June 13, 1964, from Yaeger's Slough. Of eight eggs incubated, one hatched on June 19 and another on June 23. One juvenile bird died on July 9 before exposure to infective larvae could be conducted. On August 7, the remaining bird was experimentally

fed a naturally infected dragonfly naiad which contained a larval S. tenuicirrus. Three days later this laboratory grebe died of unknown causes. A postmortem examination revealed the presence of a single immature S. tenuicirrus. This cestode (Figure 29) was 3.2 mm long and consisted of 23 segments. No internal organs were yet present.

The final feeding experiments attempted during this study were made during the summer of 1965. A very young grebe, estimated to be 2 or 3 days old, was hand caught in Jemmerson Slough on July 9. Three consecutive exposures with larval S. tenuicirrus, from the hemocoel of three naturally infected Anax junius naiads, successfully produced infections in this bird. The first larva of S. tenuicirrus within the intermediate host was fed on July 13, a second on July 20, and a third was fed four hours before the grebe was sacrificed on July 27. From this experimentally infected grebe, two adult S. tenuicirrus were recovered from the small intestine. One mature worm measured 18 mm in length and consisted of 105 segments. The other adult worm was immature. It was 5 mm long and the strobila consisted of 42 proglottids. This cestode, believed to be 7 days old, did possess testes in the posterior segments.

A single larval S. tenuicirrus was recovered from the ventriculus of the bird. The dragonfly naiad harboring the strobilocercoid and the outer sac of the latter had been com-

pletely digested during the four-hour period within the digestive tract of the definitive host. However, the inner membrane of the strobilocercoid remained intact and undamaged. It appeared white, fluid filled, and the scolex and strobila remained invaginated within the inner membrane. The length of the entire larva was 16 mm; the scolex and strobilate portion measured approximately 3 mm long with 18 proglottids present.

These feeding experiments, summarized in Table 7, definitely establish the manner in which pied-billed grebes acquire infections of S. tenuicirrus. Wetmore (1924), studying the feeding habits of P. podiceps, examined 180 stomachs and reported the contents as 46.3% insects, 27% crayfish, 24.2% fish, 4.1% other crustaceans, and 2.1% miscellaneous animals. During the warmer months of May to October, dragonflies and damselflies represented 8% of the stomach contents. In July and August, these two insects form a considerably greater part of the food; for in 19 stomachs representing these two months, their remains amounted to 34%. The greater part were naiads of dragonflies, as damselflies figured in the food of only one bird. During my investigations, 41 stomachs of P. podiceps showed many dragonfly naiads, other insects, fish, amphibians, crayfish, other crustaceans, mollusks, spiders, and aquatic plants. Feathers also formed a major part of the stomach contents.

Table 7. Summary of experimental infections of pied-billed grebes fed strobilocercoids of S. tenuicirrus within naturally infected Anax junius

Date	Number of grebes exposed	Number of infected dragonfly naiads fed each grebe	Age of worm recovered	Results
1963				
August 26	1	1	33 days	1 gravid adult 33 mm long; 162 proglottids
1964				
August 7	1	1	3 days	1 immature tapeworm 3.2 mm long; 23 proglottids
1965				
July 13		1	14 days	1 mature adult 18 mm long; 105 proglottids
July 20	1	1	7 days	1 immature tapeworm 5 mm long; 42 proglottids
July 27		1	4 hours	1 larval cestode, length of entire larva 16 mm; scolex and strobila portion 3 mm long; 18 proglottids

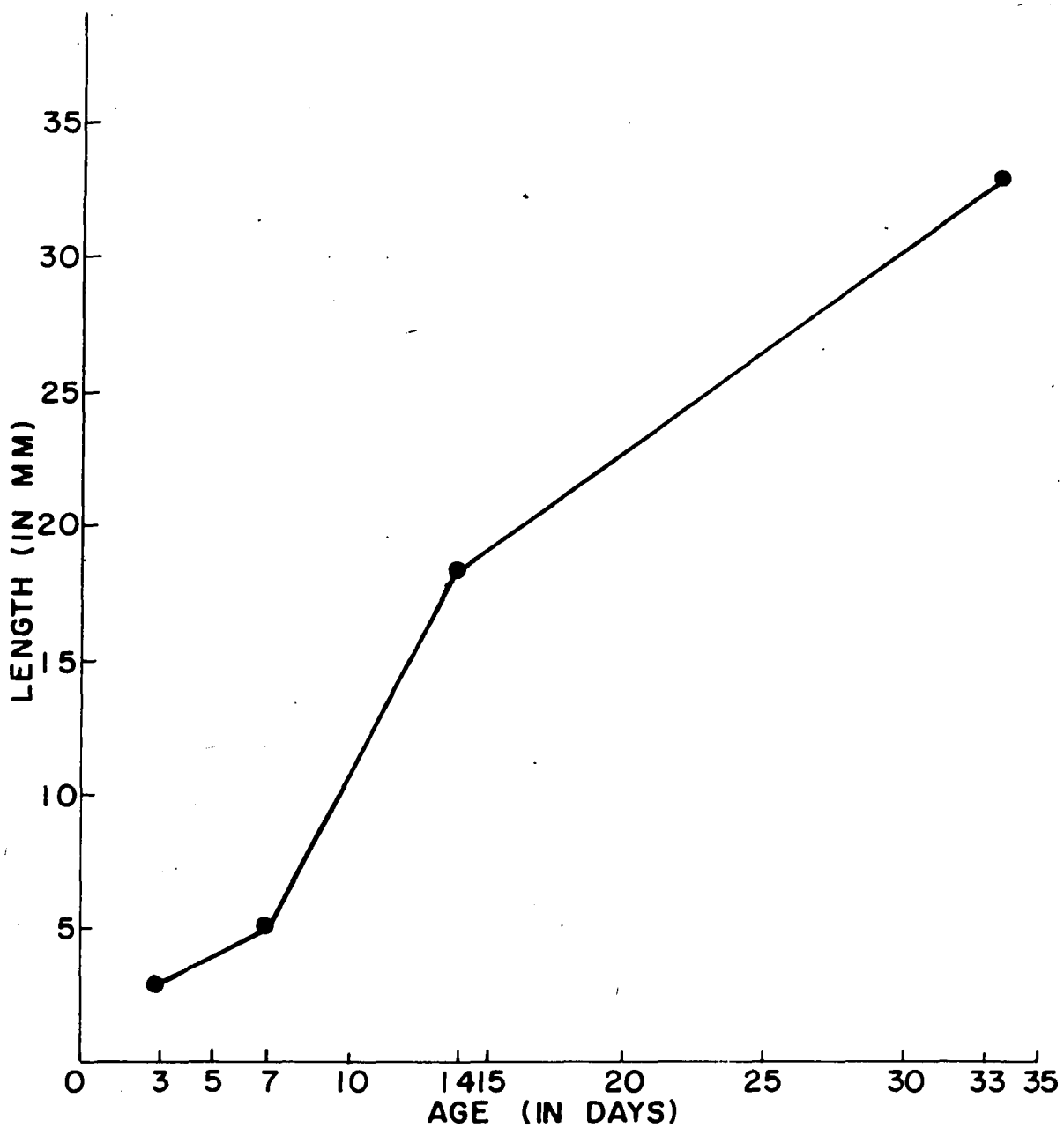
In addition, the final experimental feeding established the fact that successive exposures of the definitive host to the strobilocercoid of S. tenuicirrus do not appear to result in any type of immunity to reinfection. This is supported by natural infections of S. tenuicirrus of varying size and age within wild pied-billed grebes.

One of the striking characteristics of S. tenuicirrus illustrated by these experimental studies is the rapid increase in total body length at various ages (Graph 2).

As indicated by Wardle and McLeod (1952), linear length, commonly used as a criterion of growth, while applicable to small tapeworms such as hymenolepids is less applicable to large tapeworms. Wardle and Green (1941) studied the growth of Dibothriocephalus latum in dogs, and their conclusions were based on a comparison of the average weights of worms recovered from a series of 10 dogs killed at 3-day intervals from 3 to 30 days. They concluded that little growth of D. latum occurred during the first 6 days; from the 6th to the 15th day growth was exponential, and following maturation about the 18th day, the weight fluctuated rhythmically because of periodic egg production and apolytic loss of exhausted proglottids.

Archer and Hopkins (1958) studied the rate of growth of Diphyllobothrium sp. in the small intestine of a rat. The growth rate was calculated by dividing the average weight of

Graph 2. Increase in length of S. tenuicirrus in the definitive host


35
30
25
20
15
10
5
LENGTH (IN MM)
0
3
5
7
10
14
15
20
25
30
33
35
AGE (IN DAYS)

the worms recovered, by the average weight of the pleurocercoids fed to albino rats. Archer and Hopkins concluded that the growth pattern of Diphyllobothrium sp. consisted of three phases. An initial lag phase, during which there was a loss in weight, was followed by an interphase period of accelerating growth rate. By the 60th hour, a maximum rate of growth was reached and remained constant until the 6th day. On the 7th day, another interphase period was passed through during which growth decelerated. Only a few results were obtained for the third phase, when the growth rate was much slower after the 7th day, and fully formed eggs had appeared in the uterus.

Takahashi (1959) described the development of Diphyllobothrium mansoni in the dog. The bothria began to differentiate within 24 hours. After 96 hours, reproductive structures within the segments began to appear, and in 172 hours worms had developed to the stage of oviposition.

Studies by Penfold et al. (1937) indirectly demonstrated the rate of growth of Taenia saginata, by counting the number of proglottids shed per day by fully developed worms. They found an increase of 9-12 proglottids per day and a loss of 8-9 proglottids during the same period.

Studies on the growth of the adult phase of Hydatigera (Taenia) taeniaeformis were published by Hutchinson (1959). He found that the strobilocercus of H. taeniaeformis sheds a

portion of its strobila on entering the intestine of the cat. In his paper, the fraction of the strobilocercus which survives is referred to as the true larval strobila, and that which is shed, the pseudostrobila. During the present investigation, no shedding of the strobilocercoid of S. tenuicirrus was noted.

Hutchinson (1959) further reported that H. taeniaeformis commenced growth immediately after shedding the pseudostrobila, with no intervening lag period. Growth was based on a comparison of the estimated weight of the infecting worms after 24 hours in the cat, with the actual weight of the worm observed at a later date. His figures indicated that there were two distinct exponential phases of adult growth. During the first phase, the worms doubled their weight in 8 days, and this phase terminated on the 18th day of infection. After a short transition period which coincided with the commencement of egg production, there was a second phase of exponential growth with a doubling time of 16 days. Fully developed hexacanth embryos were not observed until the 33rd day.

Chandler (1939) found a very slow linear growth in Hymenolepis diminuta during the 5-day to 7-day period in the host, as compared with rapid growth during the 7-day to 18-day period. The rapid linear growth of S. tenuicirrus after the first 7 days in its host is clearly illustrated by

Graph 2.

Schiller (1959) provided data for the growth, development, and reproduction of the strobilate phase of H. nana in different mammalian host species. He observed in worms, from indirect experimental infections in albino mice, that the normal development of the adult involved a rapid growth in the first 10 days, maintenance of size between 10 and 14 days, and progressive senescence with decreasing size after 14 days. Comparisons made of H. nana infections in albino mice, albino hamsters, and gray squirrels indicated that growth rate, strobila size, and egg productivity vary according to the host-species.

Berntzen (1961) successfully cultured in vitro the cestode H. diminuta from the cysticercoid stage in beetles to adults. He found little change in length between the 3rd and 4th day; however, between the 4th and 15th day, there appeared a sharp daily increase of total length.

The growth of the rat tapeworm, H. diminuta, was also studied during the first 5 days in the final host by Goodchild and Harrison (1961). They determined that length increased by 1.3 times the first day, 2.6 times the second day, 3.4 times the third day, 3 times the fourth day, and 2.6 times on the fifth day. The rate of growth, therefore, reached a maximum on the third day during which the length was more than tripled. However, Chandler (1939) indicated

that the fifth day represents the most productive of the first 5 days in the host, with a total elongation four times that of the length of H. diminuta on the fourth day.

The growth of S. tenuicirrus shows a greater similarity to that of the cestode genus Hymenolepis. In the present investigation, S. tenuicirrus increased only a few proglottids the first three days. During the next four days, there was an increase of approximately five proglottids per day. This was immediately followed by a rapid development of 9-10 proglottids per day during the next 7 days. The rate of growth then began to lag, and only about three proglottids per day were produced during the next 19 days.

Pathology

Attachment of S. tenuicirrus to the intestinal lining of its definitive host produces striking pathological modifications. In most instances, the scolex is deeply embedded in the submucosa of the small intestine, and appears very similar to that illustrated by Baer (1940) in his study of S. scolopendra. The anterior end of the scolex, bearing 26 rostellar hooks, is in direct contact with the muscularis externa. Inflammatory reactions and loss of blood by the host occur in the immediate vicinity of the scolex. Frequently the mucosa, submucosa, lamina propria, and muscularis mucosae are destroyed. Worms embedded in this way can be re-

moved only by tearing the host tissue to free the hooks.

In some instances, a most unusual pathological situation was observed. In only two naturally infected grebes, large amber-colored vesicles, arranged in 4-partite or tetrad formations, appeared on the surface of the small intestine (Figure 31). Each tetrad, averaging 15 mm x 15 mm, consists of four vesicles, and marks the point of attachment of a single worm. In one instance a group of 12 vesicles (15 mm x 24 mm) was observed (Figure 32). Upon dissection, three gravid S. tenuicirrus (Figure 33) were seen, so deeply embedded that the rostellum of each could not be removed without its destruction. Sections of such deeply embedded scoleces demonstrated a unique modification of the rostellar region.

Each tetrad consists of four greatly swollen areas of the rostellum which have invaded the host tissue, producing inflammatory reactions of such magnitude that the host tissue layers are difficult to identify. The fluid-filled vesicles, however, as seen in section, are embedded in the muscularis externa, but do not separate this region from the serosa. Figure 34 illustrates the great extent of the penetration of only one of the four vesicles comprising each tetrad. Closer study of worms in situ indicates that suckers do not penetrate as deeply as does the rostellum (Figure 35). When the rostellum is withdrawn (Figure 5), a collar-like area appears

external to the suckers. This collar-like region appears also in worms in situ (Figures 35, 36) and may be easily mistaken for suckers. The latter, however, are much smaller and in sections (Figure 35) appear anterior to the collar. In Baer's (1940) illustration (his Figure 31) of S. scolopendra, what apparently have been drawn as suckers, very probably constitute this collar-like region.

Muscles of the rostellum (Figure 35) are clearly seen extending into the fluid-filled rostellar vesicles. The center of each edematous vesicle (Figure 34) appears to be filled with a finely granular fluid. Peripherally, each vesicle appears to consist of a hydropic parenchyma (Figure 35). Scattered muscle fibers are clearly seen near the base of each vesicle.

The presence of rostellar glands in S. macrorhyncha was noted by Cohn (1900) and in S. scolopendra by Baer (1940). Such glands are clearly seen in sections of S. tenuicirrus (Figure 7), but were reported by Chandler (1948) and Johri (1959) to be lacking in S. macrocirrus and S. indica, respectively. Rostellar glands have also been observed in pseudophyllidean, tetraphyllidean, and other cyclophyllidean tapeworms. Smyth (1964a, b) has recently reviewed the occurrence and cytochemistry of these glands in cestodes, and has suggested that in Echinococcus granulosus, they may serve a three-fold function. Possibly, they are hormonal and may in-

fluence growth; they may produce proteolytic secretions of nutritional significance to the cestode, or may be responsible for immunity to reinfection. In S. tenuicirrus, the latter is unlikely, since evidence reported in an earlier section of this thesis demonstrates that grebes are not immune to reinfection. A further study of these glands of S. tenuicirrus, and their possible relationship to the enormous rostellar vesicles, is certainly warranted.

Host Specificity

According to Baer (1952), Schistotaenia shows a very high degree of host specificity in that it occurs exclusively in grebes. However, Chandler (1948) reported that Dr. Robert Rausch, at the University of Wisconsin, recovered a single specimen of S. tenuicirrus from a crow, Corvus brachyrhynchos, taken near Marysville, Ohio. The present investigation included experimental exposures of mature larvae of S. tenuicirrus to bronzed grackles, Quiscalus versicolor, and mallard ducks, Anas platyrhynchos, to determine if the host specificity of this tapeworm is ecological rather than phylogenetic.

Two laboratory-raised female mallard ducks were each force-fed a single dragonfly naiad containing a mature strobilocercoid of S. tenuicirrus. The first feeding experiment involved a 10-week-old bird exposed on September 20, 1964.

This mallard was killed and examined for cestodes on October 21, 1964. No parasites were found. A similar experimental feeding, using a 13-month old female mallard, was conducted on July 26, 1965. Two weeks later, this bird was examined for helminths and results were also negative.

Since wild adult grackles were readily available, two experimental feedings with larvae of S. tenuicirrus were undertaken during the month of August in 1964 and 1965. Two mature male bronzed grackles were each force-fed naturally infected dragonfly naiads. These birds were sacrificed and examined after 2 and 3 weeks post-feeding, and no S. tenuicirrus were recovered.

Although these experiments were not numerous, they do support previous investigations indicating a high degree of host specificity for Schistotaenia.

DISCUSSION

This study on the life cycle of Schistotaenia tenuicirrus provides additional evidence for grouping members of the genus and Tatria (as suggested by Johri (1959)) within the subfamily Schistotaeniinae. In both genera, larval development is strikingly similar. These two cestodes are enclosed within similar larval envelopes, employ similar intermediate hosts and definitive hosts.

Further evidence is provided by certain morphological features of adult worms. In contrast to Amabilia, the other genus of the family, both Schistotaenia and Tatria have single male and female genital organs in each segment. Both genera have bilobed ovaries which are larger than the vitelline glands; true vaginae are absent. The seminal receptacles consist of thin-walled sacs in the midline which communicate with one another in consecutive segments.

The study of numerous specimens, furthermore, casts serious doubt upon Lopez-Neyra's (1953) statement that the family Amabiliidae is an unnatural group based on teratological specimens.

Baer (1940) suggested that the genera Tatria and Schistotaenia should be united. However, the unusual strobilocercoid larva of S. tenuicirrus provides additional evidence for considering them as distinct genera. Although

studies by Mrazek (1927) on larvae presumed to be those of Tatria (from naturally infected damselflies), have indicated certain developmental features common to both genera, the occurrence of a strobilate larva in Schistotaenia differentiates it from any known larvae of Tatria.

This study necessitates certain changes in the generic diagnosis of Schistotaenia, as presented by Yamaguti (1959), and hence is modified as follows (changes or additions indicated by underscoring):

> Of the family Amabiliidae. Small forms. Scolex may be spinose; rostellum muscular, with a single crown of bifurcate hooks whose guard is very strong; suckers may be spinose. Proglottids with marginal extensions on each side. Inner longitudinal muscles strongly or rather weakly developed. One set of genitalia per proglottid. Excretory stems of three pairs. Testes usually numerous but sometimes reduced in number, situated dorsally, extending whole width of proglottid near its posterior border. External seminal vesicle present. Cirrus pouch strongly or weakly developed; cirrus usually spinose. Male genital pores alternating regularly or irregularly. Ovary more or less lobed but not dendritic, elongated transversely and occupying whole width of medulla with a median vitelline gland behind. Uterus a transversely elongated sac; eggs rounded. Vagina passing between two excretory stems, but ending blindly near cuticle; accessory dorsoventral duct arising from median seminal receptacle and opening on both surfaces. Parasites of grebes.

S. tenuicirrus was initially named and described by Chandler (1948). The specific diagnosis is modified (changes and additions indicated by underscoring) as follows:

Total length of gravid worms, 20 to 96 mm with a maximum number of proglottids being about 200. Strobila with approximately parallel sides for most of length; maximum width exclusive of lateral appendages from 1.4 to 3 mm, the appendages reaching a length of 0.7 to 1 mm, but seldom extending straight out from worm. Scolex large, 0.6 to 1.8 mm long and 0.9 to 1.6 mm broad across the posterior margin; partly spinose. Muscular rostellum 0.4 to 0.46 mm long. Finely spinose suckers, spherical or oval, measuring 0.24 to 0.3 by 0.3 to 0.39 mm. Hooks number 26. Hooks 135 to 142 microns long, base 115 to 125 microns, 52 to 59 microns from blade to guard, and 84 to 86 microns from anterior margin to tip of guard. Cirrus pouch 0.15 to 0.24 mm long, and 0.09 to 0.11 mm broad, filled with coils of cirrus. Testes numbering 21 to 26 on poral side and 24 to 32 on aporal side. Length of cirrus about 1.5 mm; densely spined at base and bearing minute spines on flagellum. Diameter of cirrus about 30 microns at base. Maximum width of seminal receptacles 0.15 mm. Ovary densely lobulated, extending across the anterior width of the proglottid. Maximum width of ovary 1.4 mm. Vitelline gland located medially and posterior to ovary, and measuring 0.2 to 0.35 mm in greatest diameter. Eggs and oncospheres spherical, 87 to 93 microns and 50 to 53 microns in diameter, respectively. Six embryonic hooks present. Strobilocercoid larvae developing in dragonfly naiad, Anax junius.

SUMMARY AND CONCLUSIONS

1. Life cycle stages of the cyclophyllidean cestode, Schistotaenia tenuicirrus Chandler, 1948, have been studied in naturally and experimentally infected hosts during 1963-1965. Pied-billed grebes (Podilymbus podiceps L.) from the Okoboji region of northwestern Iowa served as definitive hosts.
2. Comparative measurements, structural differences, principal hosts, and geographic distribution of the adult worms of the six known species of Schistotaenia are presented.
3. Experimental feedings indicate that an incubation period of five days is necessary before eggs of S. tenuicirrus are viable. The first detailed descriptions of the egg and oncosphere of S. tenuicirrus are included.
4. Oncospheres of S. tenuicirrus develop into strobilocercoid larvae, lying free within the hemocoel of the dragonfly naiad, Anax junius.
5. Natural infections with the strobilocercoid of S. tenuicirrus were found only in the dragonfly naiad, Anax junius Drury. Infected dragonflies are never found during the months of May or June, but may appear during July through October. The average rate of infected naiads for the months of August, September, and October

during 1963-65 was 5.8%. Strobilocercoids do not appear to survive within their host during the winter months.

6. Experimental studies indicate that the strobilocercoid does not interfere with normal growth and development of the dragonfly naiad until the final instar, at which time death of the host occurs.

7. Damselfly naiads, water boatmen, backswimmers, water scuds, crayfish, and dragonfly naiads were exposed to the embryonated eggs of S. tenuicirrus; infections occurred only in Anax junius. During five feeding experiments, 11.8% of these insects acquired infections. Ingestion of gravid proglottids by dragonfly naiads does not produce infections.

8. Dragonfly naiads younger than the 9th instar do not appear to harbor strobilocercoids of S. tenuicirrus.

9. Hatching of the oncosphere and its penetration of the dragonfly intestinal wall were not seen. However, young cestode larvae were found within the hemocoel of experimental hosts 48 and 72 hours post-exposure. It was not determined if the cestode eggs entered the dragonfly naiad by means of the mouth or the rectal tracheal gills.

10. The strobilocercoid is a new form of cysticercoid larva. The origin of its various membranes was not determined in this study, but warrants additional

examination. Fully formed, experimentally developed strobilocercoids (averaging 20 mm x 3 mm) occur 28 days post-exposure. The scolex and strobila of these larvae closely resemble the youngest adult S. tenuicirrus recovered from the intestine of naturally infected grebes.

11. Grebe infections are very seasonal. Sexually mature birds harbor gravid S. tenuicirrus in May, but appear to lose these worms at the time of the nesting season or soon thereafter (July). During July, adult grebes were negative or contained only immature and mature worms. Juvenile birds have a higher percentage of infections than do adults. Of 26 juveniles examined, 24 (92%) were infected with S. tenuicirrus compared to 9 (52.9%) parasitized adult grebes of 17 examined.

12. The average number of S. tenuicirrus per infected bird was six. The greatest number of worms recovered from a single grebe was 36.

13. Experimental feedings of naturally infected dragonfly naiads to laboratory-raised pied-billed grebes clearly establish the manner in which these birds acquire infections of S. tenuicirrus. Gravid worms develop within 33 days post-exposure.

14. Experimental and natural infections of the definitive host with the strobilocercoids of S. tenuicirrus establish the fact that successive exposures to the cestode

larvae do not appear to result in any type of immunity to reinfection.

15. The growth rate of adult S. tenuicirrus within its host is slow during the first three days; however, during the next 4 days there is an increase of approximately five proglottids per day. Rapid linear growth of 9-10 proglottids per day follows the first 7 days in the host. The rate of growth then declines to about three proglottids daily during the next 19 days.

16. Scoleces of S. tenuicirrus are deeply embedded in the submucosa of the duodenum or the proximal end of the jejunum of their avian host. Considerable destruction of host tissue is apparent.

17. In some instances, adult worms are tenaciously attached by the extrusion of large vesicles formed as modifications of the rostellum. These penetrate the host's gut, and lie embedded in the muscularis mucosae.

18. Experimental exposures of mature larvae of S. tenuicirrus to bronzed gracles and mallard ducks produced no infections, thus supporting previous investigations indicating a high degree of host specificity for this cestode.

19. The genus Schistotaenia is recharacterized, and the specific diagnosis of S. tenuicirrus formulated by Chandler (1948) is modified.

LITERATURE CITED

Alicata, J. E. and E. Chang. 1939. The life history of Hymenolepis exigua. Journal of Parasitology 25: 121-127.

Archer, D. M. and C. A. Hopkins. 1958. Studies on cestode metabolism. III. Growth pattern of Diphyllobothrium sp. in a definitive host. Experimental Parasitology 7: 125-144.

Baer, J. G. 1940. Some avian tapeworms from Antigua. Parasitology 32: 174-197.

_____. 1952. Ecology of animal parasites. Urbana, Illinois, University of Illinois Press.

Berntzen, A. K. 1961. The in vitro cultivation of tapeworms. I. Growth of Hymenolepis diminuta (Cestoda: Cyclophyllidea). Journal of Parasitology 47: 351-355.

Boertje, S. B. and M. J. Ulmer. 1965. Life cycle stages of the cestode, Schistotaenia tenuicirrus Chandler, 1948 (Cyclophyllidea: Amabilliidae). Journal of Parasitology 51: 60.

Braun, M. 1894-1900. Vermes. IB: Cestodes. In H. G. Bronn. Klassen und Ordnungen des Thierreichs 4: 927-1731.

Calvert, P. P. 1934. The rates of growth, larval development and seasonal distribution of dragonflies of the genus Anax (Odonata: Aeshnidae). Proceedings of the American Philosophical Society 73: 1-70.

Chandler, A. C. 1939. The effects of number and age of worms on development of primary and secondary infection with Hymenolepis diminuta in rats, and an investigation of the true nature of "premunition" in tapeworm infections. American Journal of Hygiene 29: 105-114.

_____. 1948. New species of the genus Schistotaenia, with a key to the known species. Transactions of the American Microscopical Society 67: 169-176.

Clerc, W. 1907. Notes sur les cestodes d'oiseaux de l'Oural. III. Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, Originale 43: 703-708.

Cohn, L. 1900. Zur Anatomie der Vogelcestoden. Part I. Zeitschrift für wissenschaftliche Zoologie 67: 255-290.

Fuhrmann, O. 1907. Bekannte und neue Arten und Genera von Vogeltaenien. Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, Originale 45: 516-536.

Golikova, M. N. 1960. Biology of some species of tapeworms of aquatic birds. Akademii Nauk SSSR, Doklady 131: 287-288.

Goodchild, C. G. and D. L. Harrison. 1961. The growth of the rat tapeworm, Hymenolepis diminuta, during the first five days in the final host. Journal of Parasitology 47: 819-829.

Hickman, J. L. 1963. The biology of Oochoristica vacuolata Hickman (Cestoda). Papers and Proceedings of the Royal Society of Tasmania 97: 81-104.

Hutchinson, W. M. 1958. Studies on Hydatigera taeniaeformis. I. Growth of the larval stage. Journal of Parasitology 44: 574-582.

_____. 1959. Studies on Hydatigera (Taenia) taeniaeformis. II. Growth of the adult phase. Experimental Parasitology 8: 557-567.

Johri, G. N. 1959. Descriptions of two Amabilid cestodes from the little grebe, Podiceps ruficollis, with remarks on the family Amabiliidae Braun, 1900. Parasitology 49: 454-457.

Jones, M. 1929. Proceedings of the Helminthological Society of Washington. Journal of Parasitology 15: 220-221.

Linstow, O. F. B. von. 1892. Beobachtungen an Helminthenlarven. Archiv für Mikroskop Anatomie 39: 325-343.

Lopez-Neyra, C. R. 1953. Consideraciones sobre Acoleidae, Amabiliidae y Nematoparataeniidae. Revista Iberica de Parasitologia 13: 119-184.

Matevosyan, E. M. and V. I. Okorokov. 1959. Two new cestode species from Podiceps ruficollis and an opinion on the genus Tatria. Trudi Vsesoyuznogo Instituta Gelmintho-logii im. K. I. Skryabina 6: 131-138 (In Russian: English summary p. 138).

Milleman, R. E. 1955. Studies on the life history and biology of Oochoristica deserti n. sp. (Cestoda: Linstowiidae) from desert rodents. Journal of Parasitology 41: 424-440.

Mrazek, A. 1927. Organisasce a ontogenie larvy druhu Tatria acanthorhyncha Wedl. Vestnik Kralovske Ceske Spolecnosti Nauk 1926: 1-13.

Mueller, J. F. 1959. The laboratory propagation of Spirometra mansonoides as an experimental tool. I. Collecting, incubation and hatching of the eggs. Journal of Parasitology 45: 353-361.

Needham, J. G. and M. J. Westfall, Jr. 1955. Dragonflies of North America. Berkeley, California, The University of California Press.

Neylans, O. S., Jr. 1952. Observations on the helminth parasites of the pied-billed grebe, Podilymbus podiceps (Linn.), from Georgia. Journal of the Tennessee Academy of Science 27: 205.

Owen, I. L. 1960. Helminthological Abstracts 29: 301.

Palmer, R. S. 1962. Handbook of North American birds. Volume I. New Haven, Conn., Yale University Press.

Pearson, T. G. 1936. Birds of America. Garden City, N.Y., Garden City Books.

Penfold, W. J., H. B. Penfold, and M. Phillips. 1937. Taenia saginata: its growth and propagation. Journal of Helminthology 15: 41-48.

Pennak, R. W. 1953. Fresh-water invertebrates of the United States. New York, N.Y., The Ronald Press Company.

Peterson, R. T. 1963. A field guide to the birds. Cambridge, Massachusetts, The Riverside Press.

Rendtorff, R. C. 1948. Investigations on the life cycle of Oochoristica ratti, a cestode from rats and mice. Journal of Parasitology 34: 243-252.

Schell, S. C. 1955. Schistotaenia colymba n. sp. from the horned grebe (Colymbus auritus L.). Transactions of the American Microscopical Society 74: 347-350.

Schiller, E. L. 1959. Experimental studies on morphological variation in the cestode genus Hymenolepis. II. Growth, development and reproduction of the strobilate phase of H. nana in different host species. Experimental Parasitology 8: 215-235.

Smyth, J. D. 1963. The biology of cestode life-cycles. Technical communication no. 34 of the Commonwealth Bureau of Helminthology. St. Albans, Herts, England.

_____. 1964a. The biology of the hydatid organisms. In Dawes, B., ed. Advances in Parasitology. Volume 2. pp. 169-219. New York, N.Y., Academic Press.

_____. 1964b. Observations on the scolex of Echinococcus granulosus, with special reference to the occurrence and cytochemistry of secretory cells in the rostellum. Parasitology 54: 515-526.

Takahashi, T. 1959. Studies on Diphyllobothrium mansoni. I. Life cycle and host specificity. Japanese Journal of Parasitology 8: 567-574. (English summary, p. 572)

Venard, C. E. 1938. Morphology, bionomics and taxonomy of the cestode, Dipylidium caninum. Annals of the New York Academy of Science 37: 273-328.

Voge, Marietta. 1960. Studies in cysticercoid histology. III. Observations on the fully developed cysticercoid of Raillietina cesticillus (Cestoda: Cyclophyllidea). Proceedings of the Helminthological Society of Washington 27: 271-274.

Voge, Marietta and D. Heyneman. 1957. Development of Hymenolepis nana and Hymenolepis diminuta (Cestoda: Hymenolepididae) in the intermediate host Tribolium confusum. University of California Publications in Zoology 59: 549-580.

Ward, H. B. and G. C. Whipple. 1959. Fresh-water biology. New York, N.Y., John Wiley and Sons, Inc.

Wardle, R. A. and N. K. Green. 1941. The rate of growth of the tapeworm, Diphyllobothrium latum (L.). Canadian Journal of Research 19: 245-251.

Wardle, R. A. and J. A. McLeod. 1952. The zoology of tapeworms. Minneapolis, Minnesota, University of Minnesota Press.

Wetmore, A. 1924. Food and economic relations of North American grebes. United States Department of Agriculture. Department Bulletin No. 1196: 1-24.

Wisseman, C. L., Jr. 1945. Morphology of the cysticercoid of the fowl tapeworm, Raillietina cesticillus (Molin). Transactions of the American Microscopical Society 64: 145-152.

Yamaguti, S. 1940. Studies on the helminth fauna of Japan. Part 30. Cestodes of birds, II. Japanese Journal of Medical Science 1: 175-211.

_____. 1959. Systema helminthum. Vol. 2: The cestodes of vertebrates. New York, N.Y., Interscience Publishers, Inc.

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PLATES

Abbreviations, used in the illustrations which follow, are explained below:

AV - opening of accessory vagina

CM - circular muscle

CP - cirrus pouch

DBV - dorsal blood vessel

ED - excretory duct

H - hemocoel

I - intestine

IC - invagination canal

IM - inner membrane

IRS - inner rostellar sac

LM - longitudinal muscle

O - ovary

ORS - outer rostellar sac

OS - outer sac of strobilocercoid

PV - prostatic (intermediate) vesicle

R - rostellum

RG - rostellar gland

S - scolex

SR - seminal receptacle

ST - strobilocercoid

SU - sucker

SV - seminal vesicle

T - testis

TR - trachea

U - uterus

V - vitellaria

Plate I

Figure 1. *Schistotaenia tenuicirrus* from intestine of pied-billed grebe, *Podilymbus podiceps*

Figure 2. Three *S. tenuicirrus* adults *in situ*, in small intestine of pied-billed grebe


2

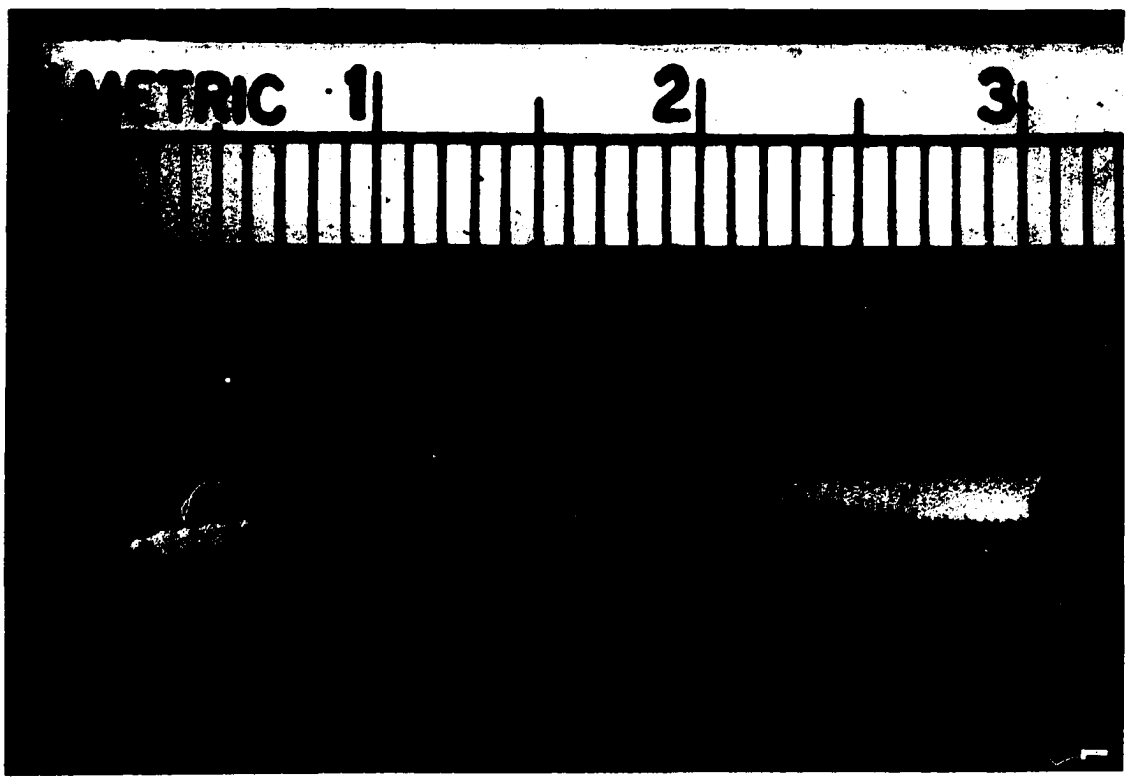
METRIC
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Plate II

Figures 3 through 5. Modifications of scoleces of *S. tenuicirrus*; all specimens from naturally infected definitive hosts

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Plate III

Figures 6 through 7. Scoleces of *S. tenuicirrus* from naturally infected definitive hosts (drawn to same scale as that shown in Figure 6)

Figure 6. Whole mount

Figure 7. Section showing details of rostellum (note rostellar gland)

Figure 8. Rostellar hook of *S. tenuicirrus*

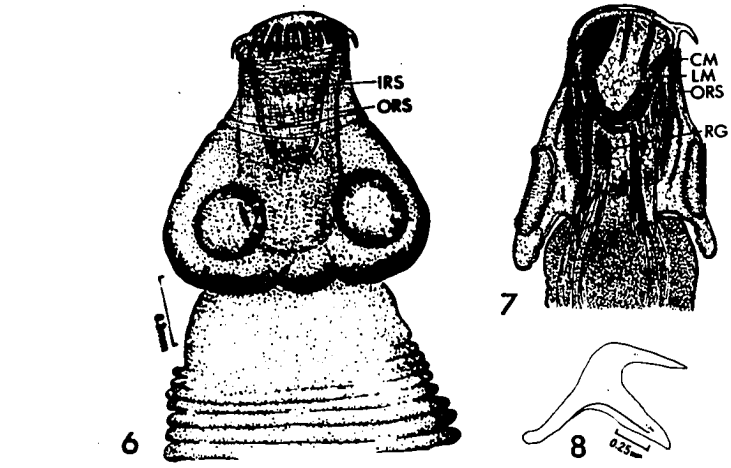
Figure 9. Cross-section through gravid proglottid of *S. tenuicirrus*

Figures 10 through 12. Proglottids of adult worms (all drawn to same scale as that shown in Figure 9)

Figure 10. Whole mount of mature segments showing testes

Figure 11. Whole mount of mature segments showing seminal vesicles, ovaries and seminal receptacles

Figure 12. Whole mount of gravid segments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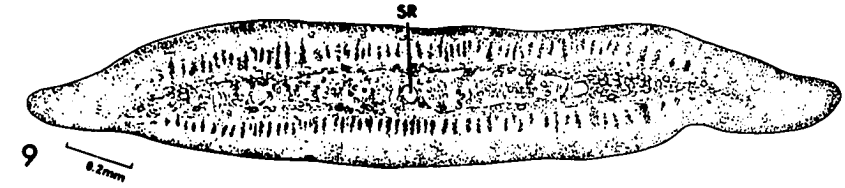
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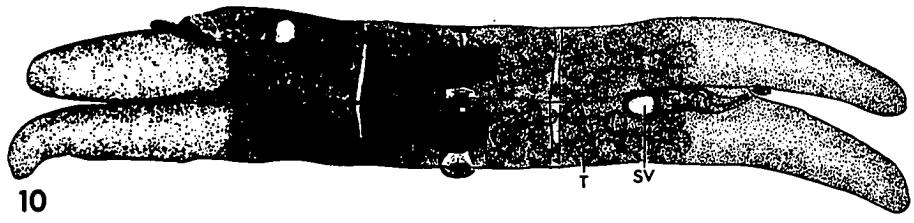
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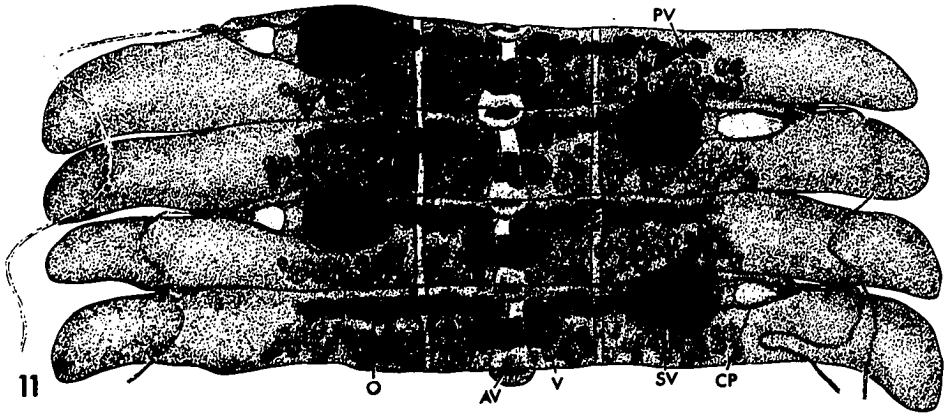
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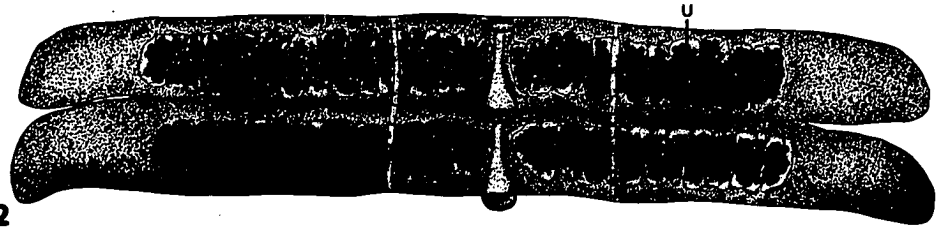
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Plate IV

Figure 13. Photomicrograph of eggs of S. tenuicirrus

Figure 14. Photomicrograph of egg containing fully developed oncosphere

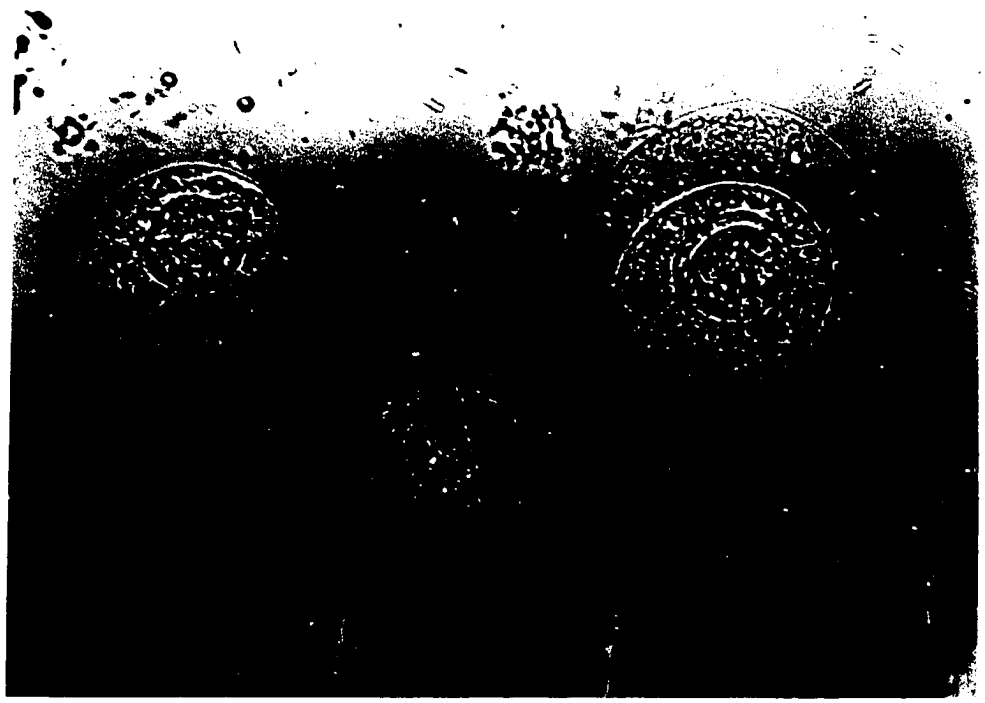

Plate V

Figure 15. Probable 9th instar of *Anax junius*

Figure 16. Dragonfly naiad of *Anax junius* (12th or 13th instar) with strobilocercoid of *S. tenuicirrus* removed from hemocoel, natural infection

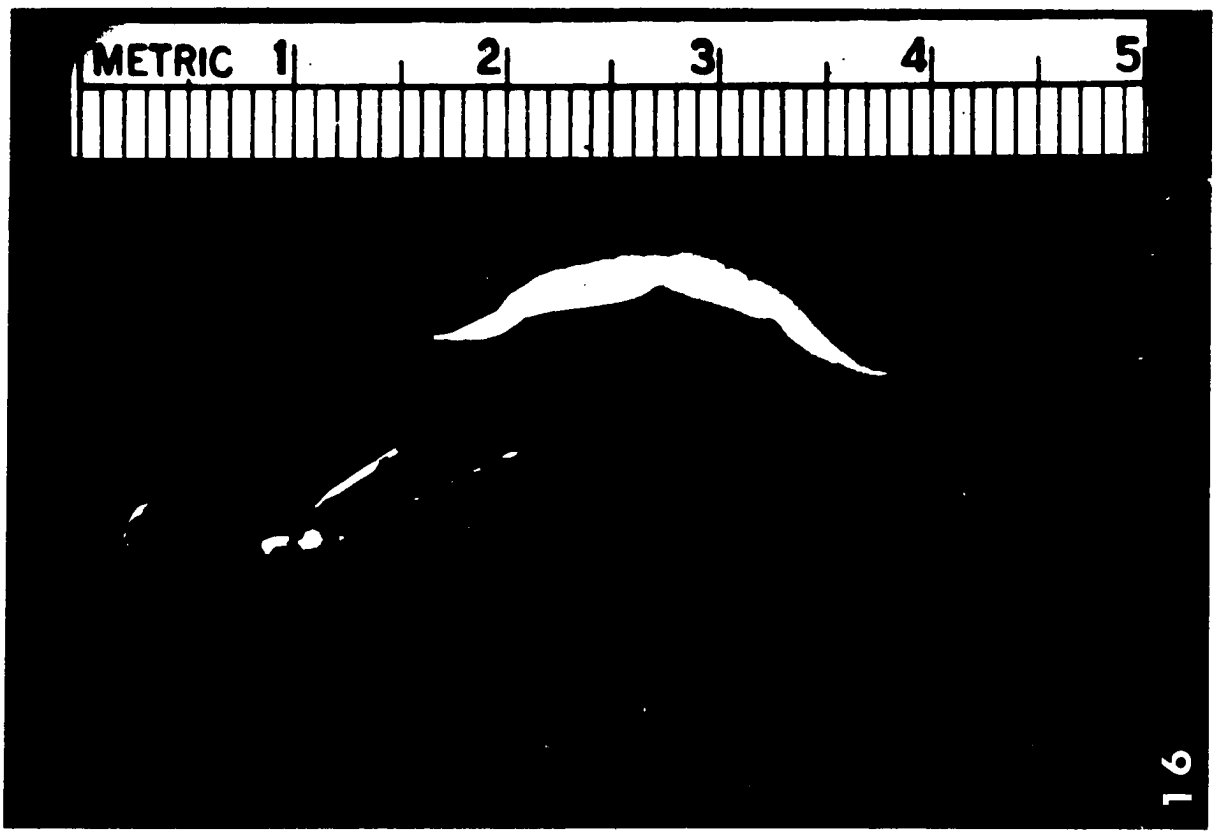
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Plate VI

Figure 17. 48-hour larva from hemocoel of experimentally infected *Anax junius*

Figure 18. Developing strobilocercoid from naturally infected dragonfly naiad, showing attachment of embryo to its enveloping outer sac

Figure 19. Experimentally developed 14-day strobilocercoid from hemocoel of dragonfly naiad

Figure 20. Anterior region of 21-day strobilocercoid showing incipient withdrawl of scolex and strobila

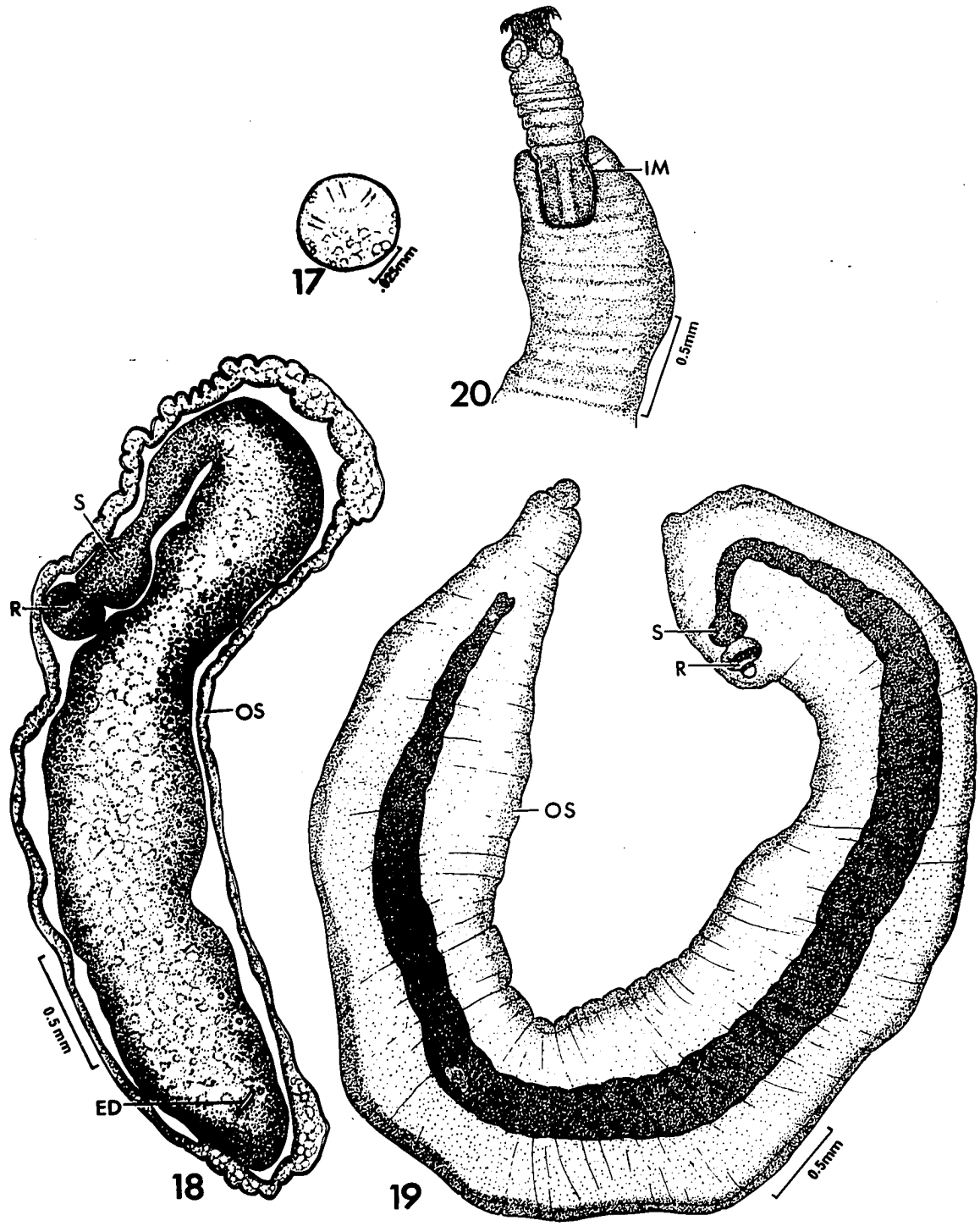
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Plate VII

Figure 21. Photomicrograph of anterior end of living 14-day strobilocercoid showing transparent outer sac

Figure 22. Photomicrograph of living, fully formed strobilocercoid of S. tenuicirrus (outer sac has been removed)

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Plate VIII

Figure 23. Fully developed strobilocercoid of *S. tenuicirrus* from hemocoel of *A. junius* (note the double membranes enclosing the embryo proper)

Figure 24. Anterior portion of strobilocercoid with outer sac removed (note the numerous papillae-like evaginations of the inner membrane)

Figure 25. Embryo proper removed from external membranes (note the distinct strobilization)

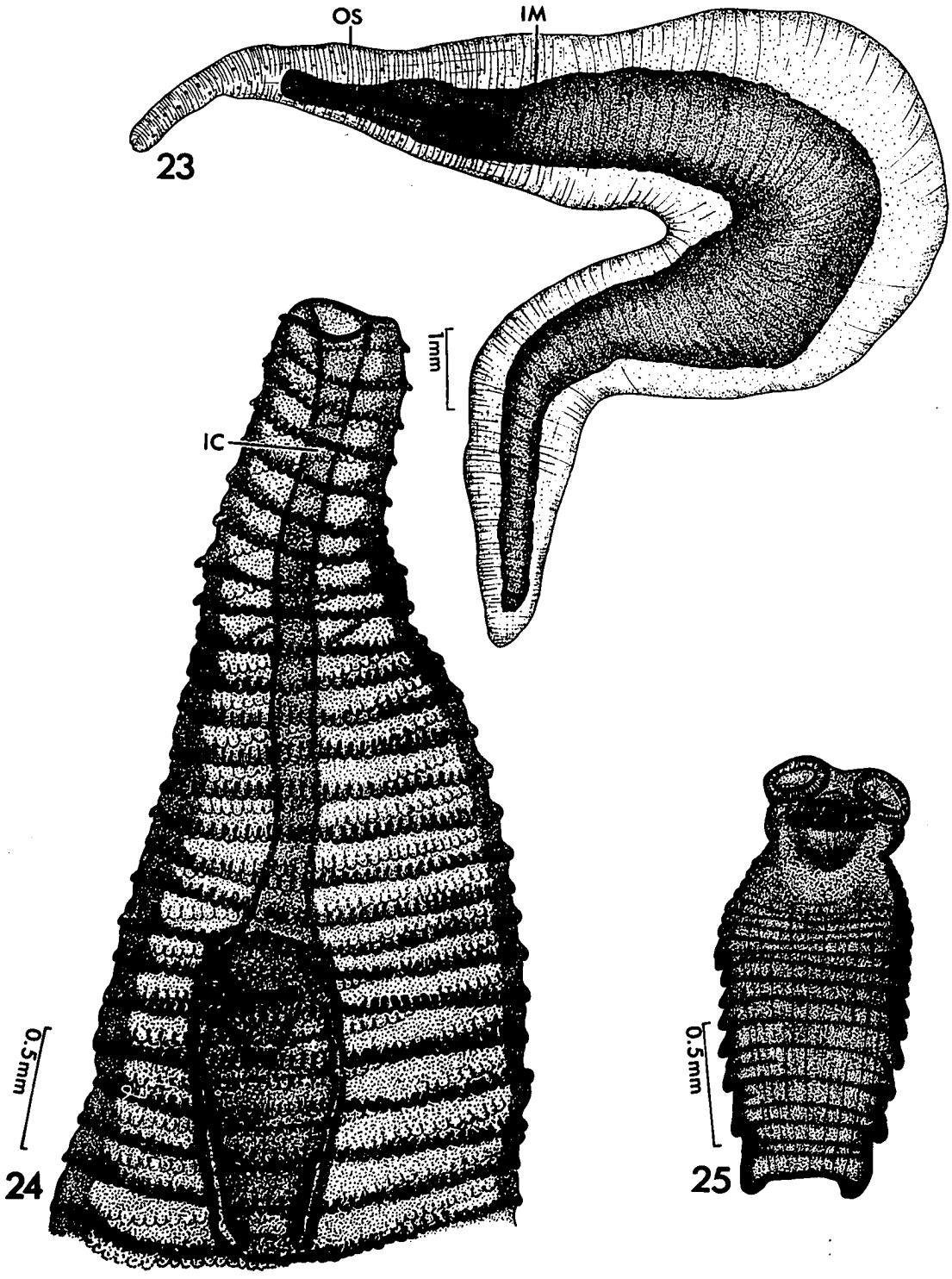
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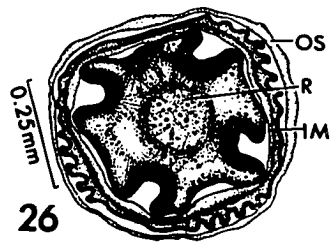
Plate IX

Figure 26. Cross-section through base of rostellar region of strobilocercoid

Figure 27. Cross-section through strobilocercoid anterior to the rostellum (note the invagination canal)

Figure 28. Cross-section through *A. junius* naiad showing a strobilocercoid within the hemocoel

Figure 29. Immature *S. tenuicirrus* recovered from the intestine of a laboratory-raised pied-billed grebe, after exposure to an infected dragonfly naiad



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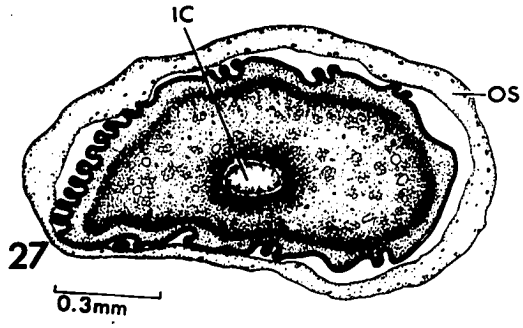

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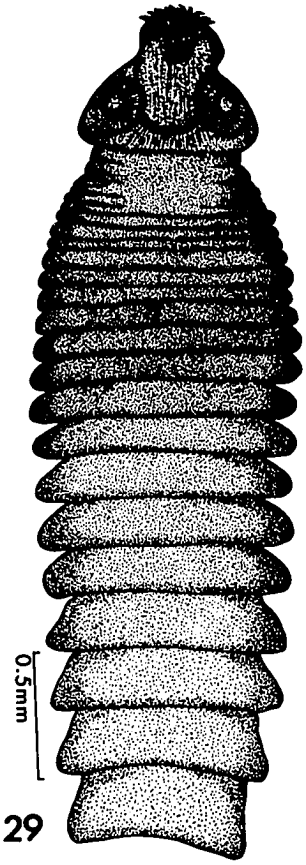

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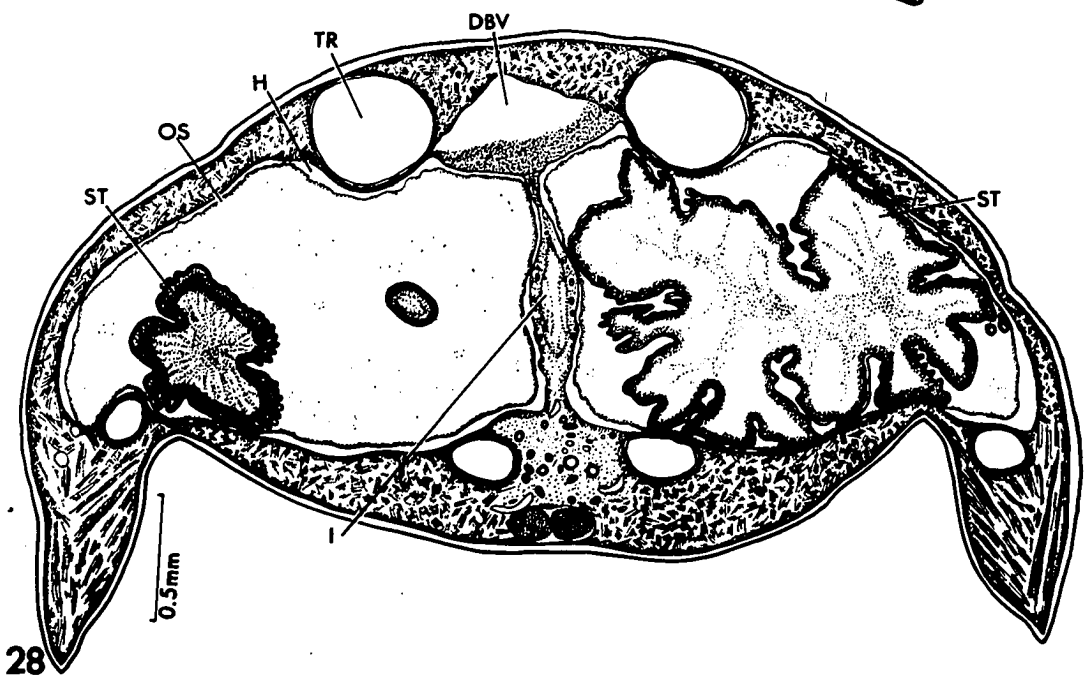

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Plate X

Figure 30. Photomicrograph of very young S. tenuicirrus recovered from intestine of a naturally infected grebe, P. podiceps

Figure 31. Surface view of intestine of pied-billed grebe showing tetrad formation resulting from attachment of S. tenuicirrus

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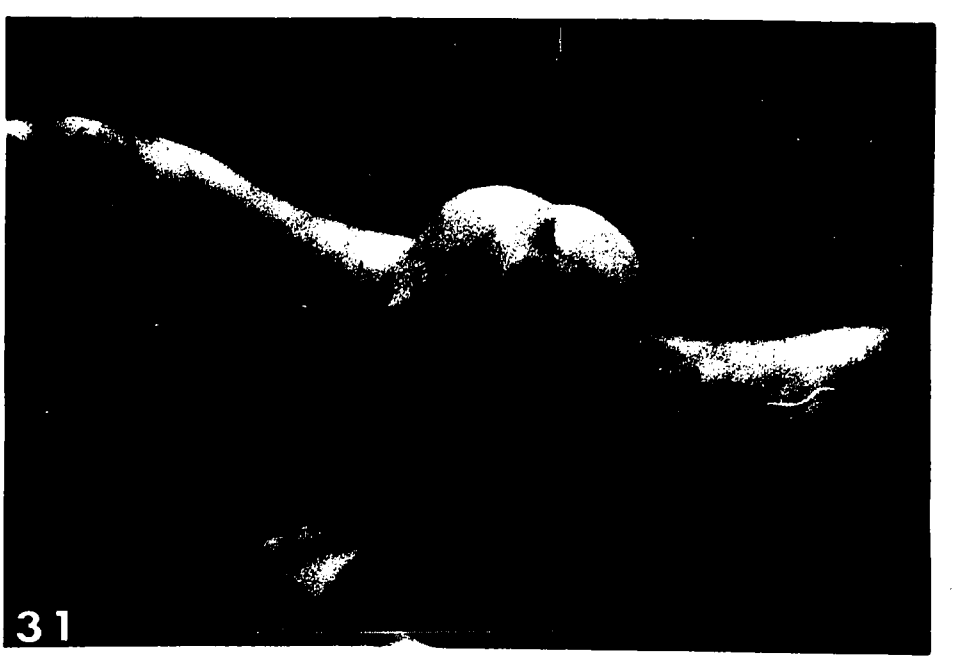

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Plate XI

Figure 32. Three tetrads, surface view, from intestine of pied-billed grebe

Figure 33. Dissected intestine showing three adult S. tenuicirrus in situ (same region of intestine as shown in Figure 32)

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Plate XII

Figure 34. Photomicrograph, low power view of adult S. tenuicirrus in situ (note extent of single large rostellar vesicle; the scolex appears at the lower right)

Figure 35. Photomicrograph of adult S. tenuicirrus in situ (note the rostellar muscle fibers extending into the rostellar vesicles and the suckers (anterior to collar))

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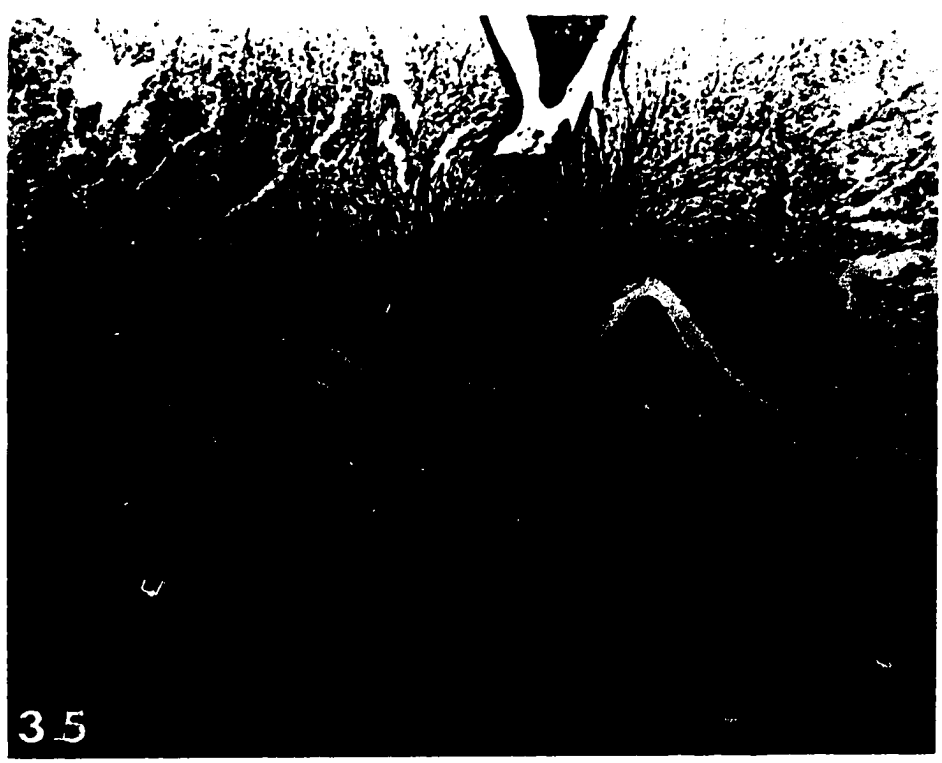

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Plate XIII

Figure 36. Photomicrograph of adult *S. tenuicirrus* *in situ*, showing rostellum

Figure 37. *Podilymbus podiceps* L., the definitive host of *S. tenuicirrus*


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